
SOCIAL BEHAVIOR, COOPERATION AND ECOLOGICAL CONSTRAINTS ON TWO CLOSELY RELATED MICE SPECIES

PhD Dissertation

Peter Mark Szenczi

Department of Ethology
Biological Institute, Faculty of Science,
Eötvös Loránd University, Hungary

Supervisor

Dr. Vilmos Altbäcker

Department of Ethology
Eötvös Loránd University, Budapest, Hungary

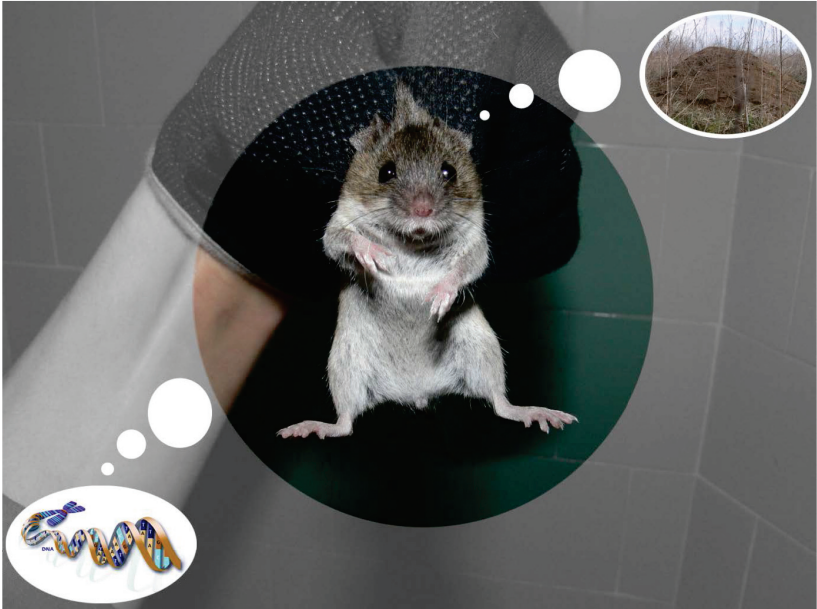
Ph.D. School of Biology

Head of PhD School: Prof. Dr. Anna Erdei

Ph.D. Program of Ethology

Head of Ph.D. Program: Prof. Dr. Ádám Miklósi

- Budapest, 2012 -



This work was supported by the GEBACO EU FP6 Grant no. 28696 to Vilmos Altbäcker. The procedures used were in accordance with the standards of the Ethical Committee for Animal Experiments at Eötvös Loránd University, the European Communities Council Directive of 24 November 1986 (86/609/EEC) and followed the guidelines of the American Society of Mammalogists.

TABLE OF CONTENTS

CHAPTER I. INTRODUCTION AND THESIS OVERVIEW	1
SOCIAL BEHAVIOR	1
COOPERATION AND CONFLICT	2
OVERWINTERING	4
BURROWING.....	6
AGGRESSION.....	7
DISPERSION	8
KINSHIP AND FAMILIARITY.....	8
THE MODEL SPECIES	10
OBJECTIVES	14
CHAPTER II. EXPERIMENTAL WORK.....	15
II/1. MORPHOLOGY AND FUNCTION OF COMMUNAL MOUNDS OF OVERWINTERING MOUND-BUILDING MICE (<i>Mus</i> <i>SPICILEGUS</i>)	16
II/2. THE CONTRIBUTION OF THE VEGETABLE MATERIAL LAYER TO THE INSULATION CAPACITIES AND WATER PROOFING OF ARTIFICIAL <i>MUS SPICILEGUS</i> MOUNDS.....	35
II/3. NATAL DISPERSAL IN TWO MICE SPECIES WITH CONTRASTING SOCIAL SYSTEMS	48
II/4. DEVELOPMENT OF THE SOCIAL BEHAVIOR OF TWO MICE SPECIES WITH CONTRASTING SOCIAL SYSTEMS	62
CHAPTER III. GENERAL DISCUSSION.....	79
CHAPTER IV. OVERALL CONCLUSIONS	84
REFERENCES	86
SUMMARY	102
ÖSSZEFOGLALÓ	103
LIST OF RELATED PUBLICATIONS	104
ACKNOWLEDGEMENTS	105

Chapter I.

INTRODUCTION AND THESIS OVERVIEW

SOCIAL BEHAVIOR

Most mammals live in groups at least for a short period during their lifetime. In general, groups form because all participants realize fitness benefits, for example better survival or increased reproductive success. Animals aggregate mainly because of the availability of certain resources or to reduce predation risk (Davies et al. 2012). Group living can improve the feeding success of an individual due to more effective defense of territories, better access to information on good feeding sites or the possibility to hunt larger prey by cooperative hunting (Galef 1991; Lindstrom 1986). Moreover, the dilution effect (decreased probability of being taken by a predator) and increased attention (Roberts 1988) improve protection against predators.

Social partners are important environmental elements since they are potential mates and participants in cooperative and competitive interactions. The formation of groups and the related behavioral patterns have both costs and benefits. Living in groups allows the development of complex social behavioral traits like alarm calls (Sherman 1977), food sharing (Benz 1993), helping (Brown 1987), communal breeding (Creel and Creel 1991), establishing dominance order (Bernstein 1981), individual recognition (Wrangham 1983) and mating systems (Emlen and Oring 1977), which further increases the gained benefit. However, there are numerous disadvantages as well; competition is higher, which leads to elevated aggression (Walters and Seyfarth 1986); while dominant individuals may monopolize resources (Wrangham 1981). The risk of infection and the threat posed by parasites are also higher (Brown and Brown 1986). Animal aggregation is expected when the costs of group-living are low or the achieved benefits are high (Krause and Ruxton 2002; Pulliam and Caraco 1984). The most fundamental form of group living is the family, where same or different-age siblings and parents live together. Family groups most often form when offspring delay reproduction and dispersion, and wait for a breeding opportunity near

their birth place (Perrin and Lehmann 2001). Although some authors do not count family as proper aggregation of animals (Alexander 1974), since the group behavior of the siblings is an aspect of their parents' investment, the company of others strongly influences individual life histories and the development of individual morphological, physiological, and behavioral phenotypes. (Hudson and Trillmich 2008).

Adaptation to different ecological conditions can result not just in markedly different physiology but contrasting social behavior (Bronson 1985). Individual differences such as environmental factors, hormonal stages and previous experience may also alter the behavior of animals in social situations.

COOPERATION AND CONFLICT

Cooperation among individuals is an omnipresent phenomenon in nature, from bacteria to primates. Cooperative behaviors appear from fairly simple to highly complicated forms. The appearance and manifestation of cooperation is closely related to the sociality of the animals. Individuals may work together in hunting and foraging, defending their lives, territory or kin from predators or conspecifics, and migrating (Dugatkin 1997). Since there are still debates about the definition of cooperation and the word is often used as the synonym of altruism, in this work I will use Dugatkin's (1997) definition as "cooperation is an outcome that - despite potential relative costs to the individual - is 'good' in some appropriate sense for the members of a group, and whose achievement requires collective action"; whereas cooperative behavior or altruistic behavior can be defined as behavior that benefits another organism, while being costly to the organism performing the behavior (Trivers 1971).

How cooperative behavior evolves is a difficulty for evolutionary theory ever since. The problem is that cheaters, individuals who do not behave cooperatively but gain the benefit from others cooperating, achieve a competitive advantage and are able to invade and take over the population. It is best described by the so called Prisoner's dilemma (Axelrod and Hamilton 1981), where both individuals would benefit from mutual cooperation but each individual would benefit more from defecting when his partner cooperated. In other words, the strategy is sensitive to cheaters. There are three main explanations on how cooperative behavior can evolve and survive in a population despite the aforementioned difficulties: kin

selection (Hamilton 1964), reciprocity (Axelrod and Hamilton 1981; Trivers 1971) and mutual benefit (Lima 1989; Maynard Smith 1983).

The theory of kin selection argues that when individuals are cooperative with their close relatives the gained inclusive fitness benefits can outweigh the temptation to defect, allowing the payoff for cooperation to exceed that of defection when a companion cooperates (Hamilton 1964). There are plenty of empirical evidences supporting this theory and it seems to play a crucial role in the evolution of eusocial insects but its potential importance in vertebrates has been much debated (Clutton-Brock 2002; Griffin and West 2002, 2003). However, in many cases when cooperation is observed in nature, kinship is by some means involved.

Cooperation can be maintained between nonrelatives if the individuals behaving cooperatively are compensated by returned benefits from others in reciprocal interactions (Axelrod and Hamilton 1981; Trivers 1971). Directed reciprocity is vulnerable to cheaters but in simulated iterated prisoner's dilemma models the 'Tit for Tat' and 'Win-stay, Lose-shift' strategies (Nowak and Sigmund 1993), can result in long term cooperation between partners. Two other, more empirical models show how long-term cooperation can be maintained: by 'partner choice' and 'partner fidelity' (Bull and Rice, 1991). Individuals may either preferentially receive benefits from others who choose them based on their traits indicating their cooperative behavior (partner choice), or their investment is reciprocated by others with coupled fitness interests (partner fidelity). However, reciprocal altruism has been rarely described in free-ranging social mammals and each case is open to alternative explanations.

This theoretical problem of cooperation is well known in economy and human morality as well, where it is called the 'tragedy of the commons' (Blaustein 1983; Hardin 1968). The benefits of cooperation in vertebrate societies may consequently show parallels with those in human societies, where cooperation between unrelated individuals is frequent and social institutions are often maintained by generalized reciprocity (Clutton-Brock 2002).

Mutual benefit models presume situations where there is no temptation to cheat because cooperating always provides the highest payoff regardless of the opponents' behavior (Maynard Smith 1983). In Lima's (1989) model the factors that fortify the tendency to cooperate are: low probability of success when alone and to replace the current partner and large number of interactions before the end of the association.

When considering the explanations for cooperation given above, it must be noted that the different mechanisms are not mutually exclusive. Although cooperation is favored between relatives, if there is a mechanism for the repression of competition between relatives, then this can lead to even higher levels of cooperation. As in case of other social behaviors, natural selection will only favor cooperative actions in certain ecological contexts. When studying cooperation it is mandatory to analyze the benefits provided to the participants in close connections with the ecological constraints they face.

OVERWINTERING

For small mammal populations living in temperate and cold climates, especially in northern latitudes, winter is an obvious bottleneck. Winter reproduction is uncommon; hence these populations usually decline during the coldest period of the year. As primary production declines along with ambient temperature, small mammals encounter an energy crisis. With the increased energy needed to maintain body temperature animals face a potential imbalance in supply and demand. Significant decrease of population size have been observed in many species, including bank voles (*Clethrionomys glareolus* - Gipps et al. 1985; Pucek et al. 1993) , short-tailed field voles (*Microtus agrestis* - Bradshaw 1992) , tundra voles (*Microtus oeconomus* - Aars and Ims 2002), yellow-necked mice (*Apodemus flavicollis* - Pucek et al. 1993) and house mice (*Mus musculus* - Berry et al. 1973). Over-winter mortality in small mammals is strongly influenced by low ambient temperatures. Metabolic rate in endotherms is related to body size (Heldmaier et al. 2004); smaller animals use proportionally more energy than larger ones because of their higher surface to body mass ratio (McNab 2008). For small endotherms, the energetic cost of maintaining constant body temperature becomes particularly high, especially during cold exposure or food shortage (Formozov 1966). This leads to a strong selective pressure for the evolution of physiological, anatomical and behavioral adaptations to increase heat production, reduce

heat loss and/or energetic needs, which enhance their probability of survival over the winter. Some of these traits are solitaire responses, affecting only the individual; others are socialistic, requiring more animals to interact.

Common ways to reduce heat loss is by increasing the heat retaining capacity of the pelage or the use of nests and/or burrows. Increased fur thickness however is only feasible by larger animals because of their more advantageous surface - body mass ratios. Smaller animals must use burrows or increase their endogenous heat production, mostly by non-shivering thermogenesis in the brown adipose tissue, which produces approximately the same amount of heat as shivering (Janský 1973) but it has a considerable energetic cost. Hibernation, the reduction of body temperature close to ambient temperature, is a well-known strategy among mammals to escape the cold season. Hibernating animals retreat into a shelter (or hibernaculum) and stay there until the next spring. During this time animals live off their body fat storages accumulated during the warmer seasons (Humphries et al. 2003). For animals that do not migrate or hibernate, other behavioral strategies, including food hoarding to satisfy nutrient demands (Vander Wall 1990), huddling in communal nests to reduce heat loss (Contreras 1984; Hayes et al. 1992), reduction in winter activity levels to avoid exposure to low temperatures (Jackson et al. 2001) are of special importance. Huddling has evolved as a social strategy for energy saving during harsh climatic conditions or at special life stages; several animals reduce their exposed surface area, thus decreasing heat loss to the environment by maintaining close body contact (Canals et al. 1989). For example, Alpine marmots use social thermoregulation during winter, while juvenile survival increases with group size (Allaine et al. 2000). Huddling at cold temperatures is also known in deermice (*Peromyscus* spp. - Howard 1951), taiga vole (*Microtus xanthognathus* - Wolff and Lidicker 1981), rats (*Rattus norvegicus* - Alberts 1978), and rabbits (*Oryctolagus cuniculus* - Bautista et al. 2003). By huddling, animals also increase nest chamber or microhabitat temperature (Glaser and Lustick 1975). With this strategy, animals can decrease the amount of food required to maintain body temperature (Springer et al. 1981). For rodent species that do not hoard food, these microclimatic conditions may counteract the physiological costs of foraging. Huddling is a universal behavior among new born and juvenile endotherms but it is also common in overwintering family groups.

Due to the aforementioned seasonal food availability many small mammals have elaborated diverse behavioral patterns of food caching to adapt to winter food shortages (Vander Wall 1990). By collecting potential nutrients to a favorable microhabitat, the animals not only reduce the time needed to forage under harsh conditions but can also protect it more efficiently. Such behavior appears in North American pikas (*Ochotona princeps* - Broadbrooks 1965; Dearing 1997), Daurian pikas (*Ochotona daurica* - Formozov 1966), Brandt's voles (*Lasiopodomys brandtii*—Zhong et al. 2007), Mongolian gerbils (*Meriones unguiculatus* - Ågren et al. 1989), and arctic ground squirrels (*Spermophilus parryii* - Zazula et al. 2006).

BURROWING

Most mammals use some kind of shelter either daily or seasonally. It can be very simple, merely a resting place or den, but many non-fossorial species construct nests and dig burrows or tunnel systems. Living or nesting in burrows is usually acknowledged to offer protection from predators and from climatic extremes (Reichman and Smith 1990). Steppes and other grasslands are much more exposed to climatic extremes than woods. For animals living in such biotopes, burrowing adaptations that provide a more suitable microclimate is considered even more important (Bethge et al. 2004; McCafferty et al. 2003).

The dry soil is an excellent insulator; therefore the burrow environment is significantly more moderate than above ground. Soil temperature fluctuates less even a few centimeters deep into the soil than on the surface (Kay and Whitford 1978; Kenagy 1973). Thus, within-burrow temperature is buffered against external temperature changes. The same can be said about other environmental factors; wind, rain and snow have lesser effect on animals when underground, and in an arid environment the burrow is more humid. Avian predators have almost no access to burrows and even though some mammals and reptiles are able to enter and chase their prey in tunnels, while some others can dig them out, it is much safer for an individual to stay underground. Hence, if an animal has no reason to be above ground (like foraging, territory defense and searching for a mate) it is expected to stay in its protective burrow. (Reichman and Smith 1990). Even mammals using the simplest burrows spend the majority of their lives in them.

The number of species using underground excavations as shelter or living entirely subterranean suggests that burrowing is advantageous. However, constructing and maintaining such a structure is expensive. The energy cost of digging is high (Ebensperger and Bozinovic 2000), also varies with soil density, burrow size and structure. According to (Vleck 1979), burrowing requires 360-3400 times as much energy as moving the same distance on the surface. But other authors argue that the cost of excavating and maintaining a simple, shallow burrow system is probably not high compared to other metabolic costs for a mammal, especially if averaged over the lifetime of the burrow since in many species the burrow system is inherited (Reichman and Smith 1990).

Most burrows contain chambers with nests made from plant material or fur. Nests further insulate small mammals from fluctuating ambient temperatures (Casey 1981). The insulation quality of nests depend on several factors, such as nest structure (McGowan et al. 2004; Redman et al. 1999), size (height, thickness, and volume - Grubbauer and Hoi 1996; Redman et al. 1999; Szentirmai et al. 2005), nest material (Mertens 1977), and its moisture content. Building the nest deep in the soil further improves its insulating capacities. Also, communal nesting further enhances the thermoregulatory function of the nests.

AGGRESSION

The term “aggression” is applied to a wide range of behaviors. In a strict sense, aggression is any behavior used with the intent to cause physical harm (Hinde, 1974). In a broader sense, it may not have the intent to cause direct physical injury but be detrimental to the opponent in other ways (Wingfield et al., 2006). In most species aggression relies on highly visual and ritualized displays which effectively channel aggression, govern conflict resolution, and structure how individuals interact (Lorenz, 1963).

Aggression plays a major role in the determination of mating systems and social organizations (Trivers 1972). Intraspecific aggression often has a major role in shaping social structure and spatial distribution of conspecifics through territorial behavior connected with competition for space, mates and resources (Grant 1972). The level of agonistic behavior and tolerance between individuals is mostly under the influence of ecological traits and in direct connection with sociality, the presence or absence of social groups, and the breeding system (Armitage 1981; Crowcroft and Rowe 1963; Getz and Carter 1980; Getz et al. 1981;

Insel et al. 1995; Livoreil et al. 1993; Patris et al. 2002; Sachser et al. 1999; Shapiro et al. 1986). Mutual tolerance between individuals is essential for carrying out cooperative behavior traits, while selective aggression usually directed toward strangers may be very important in maintaining territories and social bonds established between individuals.

DISPERSION

The movement of the individuals from their place of birth to a different habitat is under ecological and social influence (Dufty and Belthoff 2001). Dispersal is one of the basic life-history traits of a population (Clobert et al. 2004; Dingle 1996; Gadgil 1971). It influences the genetic structure, and plays a fundamental role in demographic processes (Gaines and McClenaghan 1980). Perrin and Goudet (2001) showed that the level of sociality could strongly affect natal dispersal patterns. It has also been recognized that population structure plays a decisive role in the evolution of cooperation (Nowak and May 1992). Not only because spatial aggregation is necessary for individuals to interact, but because limited dispersal (population viscosity) tends to keep relatives together (Hamilton 1964). In that case, altruism directed toward all the neighbors will be favored as neighbors tend to be relatives. This form of kin selection, involving limited dispersal, has been suggested to be important for the production of public goods (Brown 1999).

Many vertebrates show sex-biased dispersal. Mating systems of species or populations supposed to have a fundamental role in this asymmetry (Dieckmann et al. 1998). There are several hypotheses explaining the role of mating systems in natal dispersal: competition for resources (Greenwood 1980; Greenwood and Harvey 1982), competition for mates (Dobson 1982), and avoidance of inbreeding (Wolff 1993, 1994). As a general rule polygynous/promiscuous species usually display a male-biased dispersal, while both sexes disperse or dispersal is female-biased in monogamous species (Dobson 1982; Favre et al. 1997; Greenwood 1980; Perrin and Mazalov 2000; Wolff 1993).

KINSHIP AND FAMILIARITY

Kin recognition is the observable, differential behavioral response to conspecifics (Mateo 2003). Whether the differential response is based on genetic similarity or acquaintance varies among taxa and is reflected in their social systems. Field studies and laboratory data

indicate that related individuals are more tolerant towards each other than non-relatives. Rats (*Rattus norvegicus*), house mice (*Mus musculus*), white-footed deer mice (*Peromyscus leucopus*) can discriminate between unfamiliar kin and nonkin (Aldhous 1989; Barnard and Fitzsimons 1988; Grau 1982). Cactus mice (*P. eremicus*) treat familiar nonkin as kin (Aldhous 1989). Belding's ground squirrel (*Spermophilus beldingii*) full-sisters are less agonistic and more cooperative than half-sisters (Holmes and Sherman 1982). Some studies demonstrated kin recognition that is not based on prior contact (Grau 1982). Juvenile Richardson's ground squirrels (*Spermophilus richardsonii*) can recognize their biological siblings even if they were separated at birth (Davis 1982). In some cases continued exposure to kin cues is necessary to maintain recognition ability (Porter 1988). On the other hand, several rodent studies (Gavish et al. 1984; Holmes 1984; Porter et al. 1978) show the familiarity of the individuals and not the coefficient of kinship itself plays the central role in social interaction (Charnov and Finerty 1980). In Deer mice (*Peromyscus maniculatus*), familiar males displayed significantly less aggression than unfamiliar males; kinship per se generated no reduction in aggression (Dewsbury 1988). White-footed mice (*Peromyscus leucopus*) showed a significant preference for siblings reared together versus siblings reared apart, and for non-siblings reared together as compared to siblings reared apart (Halpin and Hoffman 1987).

Kin recognition

Several mechanisms for the perception component of kin recognition have been proposed (Holmes and Sherman 1982; Mateo 2003).

- Recognition via spatial cues: Animals can recognize each other indirectly, based on spatial cues. They treat every individual in a particular area (e.g., mother-offspring recognition in nest or burrow) as kin.
- Recognition via prior association: Animals may learn the phenotypes of individuals during early development (e.g., siblings and parents) and use this later to discriminate these familiar relatives from unfamiliar animals.
- Recognition via phenotype matching: Animals learn the phenotype of their own and their familiar kin, and subsequently they can compare unknown animals' phenotypes to this learned recognition template. Phenotype matching requires a correlation between phenotypic and genotypic similarity so that individuals with traits that most closely match an animal's template are its closest kin.

THE MODEL SPECIES

Adaptation to different habitats plays a more important role than phylogeny in the evolution of behavior and social systems (Carranza 2000; Emlen and Oring 1977). Even between closely related species, differences in behavior, social organizations and mating system can be significant. Hence, the comparison of closely related species is an important tool in ethology and behavioral ecology.

The monogamous California mouse (*Peromyscus californicus*) and the white-footed mouse (*Peromyscus leucopus*) is well-known example for closely related species with different mating system, and social behavior. Many comparative studies have been made on these species in the last decades (Bester-Meredith et al. 1999; Dudley 1974; Gubernick and Alberts 1987; Korytko and Vessey 1991; Metzgar 1971; Schug et al. 1992; Trainor and Marler 2001; Wolff and Cicirello 1990). The *Mus* group is even a better model for comparative behavioral studies because the phylogeny of this group is well known (Bonhomme et al. 1984; Boursot et al. 1993; Chevret et al. 2005; Sage et al. 1993), and different species of the group have been studied in various habitats.

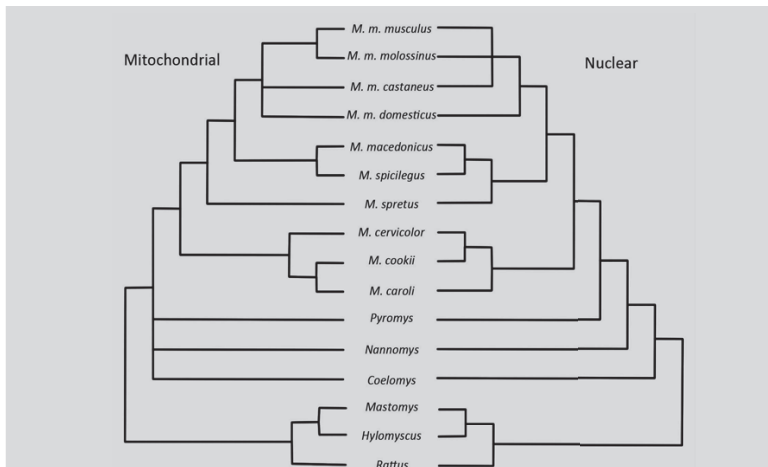


FIGURE 1/1 PHYLOGENETIC TREE OF THE GENUS *MUS*. RESULTS OF PARSIMONY ANALYSES OF COMBINED MITOCHONDRIAL DATA (CYT B AND 12S) AND COMBINED NUCLEAR DATA (SRY, B2M, ZP-3, AND TCP-1).

MUS SPICILEGUS

The mound-building mouse (*Mus spicilegus* Petényi 1882) is endemic to Hungary where it reaches its most north-eastern distribution. It can be found in the Carpathian Basin, The Balkans, Ukraine and Moldova. They inhabit various agro-ecosystem, and usually numerous throughout their range (Sokolov et al. 1998).

This species is believed to be the only one in the *Mus* complex to exhibit a socially monogamous mating system (Baudoin et al. 2005; Patris et al. 2002). They establish a strong social bond between partners (Patris and Baudoin 1998). Both sexes of the adult mice show high level of aggressiveness towards unfamiliar (Patris et al. 2002; Simeonovska-Nikolova 2003; Suchomelova et al. 1998). They form stable male-female associations in experimental groups (Baudoin et al. 2005; Simeonovska-Nikolova 2003), while polygynous females have low reproductive success (Gouat and Feron 2005). Males and females cooperate in the parental care of their offspring (Patris and Baudoin 2000).



FIGURE 1/2 DISTRIBUTION OF THE MOUND-BUILDING MOUSE, SOURCE: IUCN (2012)

The mound-building mouse is also famous for its unique communal overwintering behavior. Mice construct large mounds during autumn, in which seeds and other kinds of plant materials are cached, and they overwinter under these structures (Murariu 1981; Naumov 1940; Sokolov et al. 1998). Individuals inhabiting the mound are almost exclusively juveniles (Canady et al. 2009; Garza et al. 1997; Milishnikov et al. 1998; Poteaux et al. 2008; Simeonovska-Nikolova 2007). They might belong to a single litter but more commonly derived from differently related female parents and their unrelated mates (Garza et al. 1997). Overwintering individuals delay their sexual maturation, they only leave the mound at spring, when they disperse and reproduce (Milishnikov et al. 1998; Naumov 1940; Orsini et al. 1983; Simeonovska-Nikolova and Gerasimov 2000). The delay is the result of the social effects of living in groups and not the environmental conditions of winter (Feron and Gheusi 2003; Gouat et al. 2003a). This kind of cooperative behavior demands that juveniles must recognize and be tolerant of each other, but defend their mound, protect their resources from intruders.

The mound-building mouse - despite its unusual mating system and unique overwintering behavior - is still a barely investigated species, which makes it a promising subject of biological studies. In my comparative experiments I chose the house mouse as reference, since they are close relatives and it is the most extensively studied species in its genus.

MUS MUSCULUS

The house mouse (*Mus musculus* Linnaeus 1758) is a widespread and abundant species, also a well-known inhabitant of human settlements. In Europe it is typically commensal, and can be found in a very wide range of man-made habitats and feral areas. In some parts of its native range it maintains wild populations in outdoor habitats as well. These are referred as noncommensal. Free-ranging house mouse inhabits fields, shrub lands, sandy dunes, etc. outside buildings and urbanized areas. Among the four subspecies of the house mouse, Hungary lies in the area of the *Mus musculus musculus*.

The house mouse in Hungary is polygamous, with social group territoriality, adapted to the human environment (Bronson 1979; Brown 1953; Crowcroft and Rowe 1963; Lidicker 1976). They breed opportunistic rather than seasonal (Bronson and Perrigo 1987). They mature and reproduce early, while showing male-biased dispersal (Gerlach 1990, 1996).

Males compete for females and develop dominance-submission relationship inside their social group (Bronson 1979; Crowcroft and Rowe 1963; Lidicker 1976; Reimer and Petras 1967). Populations are maintained largely by resident territory holders aggressively chasing intruders from attractive resources. Cooperation between individuals is limited to the communal nursing of pups by females while sharing a common nest and even nurse each-others pups. Sisters are more likely to participate in such behavior. The observed characteristics of communal nursing may range from total equality to biased (Dobson and Baudoin 2002; Dobson et al. 2000; Hager and Johnstone 2007; König 1993; König 1997; Manning et al. 1995; Parmigiani 1984; Sayler and Salmon 1969).

The house mouse is the most commonly used mammalian research model with many established wild, inbred, outbred, and transgenic strains, hence many experiment protocols are described and tested extensively.

Allonursing is a highly co-operative behavior where females nursing offspring that are not their own. Packer et al. (1992) reported more than 100 mammalian species from 14 orders showing allonursing behavior. It is most common in polytocous taxa that form small groups and this is consistent with models of cooperation based on kin selection, reciprocity or mutualism.

OBJECTIVES

The aims of this study were:

1. To study the overwintering behavior of the mound-building mouse.
 - a. Assess the importance of the mounds during overwintering. Mound building is expensive for participating individuals; hence its effect on their overwintering success should be demonstrated.
 - b. Determine the role of the mounds. The mound may have several roles in the overwintering of the mound-building mice. Clarifying the benefits of the mounds may lead to a clearer conception on the ecological conditions leading to this kind of adaptation.
2. To study the social behavior of the mound-building mouse in comparison to the house mouse.
 - a. Natal dispersion plays an important role in the formation and maintenance of family groups and other social organizations. Contrasting social systems and communal overwintering implies that the pattern of dispersion is different between the house mouse and the mound-building mouse.
 - b. Selective tolerance is key in maintaining groups, in which cooperation becomes possible. Aggression within and between groups will differ in the house mouse and mound-building mouse.

Chapter II.

EXPERIMENTAL WORK

II/1. MORPHOLOGY AND FUNCTION OF COMMUNAL MOUNDS OF OVERWINTERING
MOUND-BUILDING MICE (*MUS SPICILEGUS*)

Péter Szenczi*, Oxána Bánszegi, Anita Dúcs, Csongor I. Gedeon, Gábor Markó, István Németh, and Vilmos Altbäcker,

Published in: Journal of Mammalogy

Volume 92, Issue 4, August 2011, Pages 852-860

ABSTRACT

Mound-building mice (*Mus spicilegus*) construct large mounds of soil and plant material in autumn, where juvenile animals overwinter in groups without reproducing. The mounds have several functions, including food storage, thermoregulation, and protection from predators. We examined whether these mounds have food storage or thermoregulatory function. Mice used mainly seeds and ears of certain plants (*Echinochloa* sp., *Chenopodium* spp., and *Setaria* spp., depending on availability) as building materials, but microhistological analysis of mice feces revealed that the cached plants are not represented in their actual diet. By comparing the features of soil under the mound to neighboring random points we found that the mounds have water-insulating and thermoregulatory properties. We also found a positive correlation between the size of the mound, its vegetal content, and its effectiveness to lessen the impact of the harsh conditions of the outside environment. Mound size was dependent on the number of inhabitants, indicating that larger mounds are constructed by and shelter bigger groups. The existence of communal mound building thus can contribute greatly to successful overwintering and the relatively low fluctuation in population size in this species.

Keywords: diet, *Mus spicilegus*, overwinter, thermoregulation, water insulation

INTRODUCTION

Winter food is crucial for the survival of animals in northern temperate regions where green vegetation is scarce or covered by snow during winter (Formozov 1966). Due to seasonal food availability, many small rodents and lagomorphs have elaborate behavioral patterns associated with storage of dry plant parts as winter food, and some species feed from under the snow or spend the winter in hibernation.

Small mammals at northern latitudes exhibit a diverse pattern of food caching to adapt to winter food shortages (Vander Wall 1990). Food-hoarding behavior appears in North American pikas (*Ochotona princeps* - Broadbrooks 1965; Dearing 1997), Daurian pikas (*Ochotona daurica* - Formozov 1966). Brandt's voles (*Lasiopodomys brandtii*—Zhong et al. 2007), Mongolian gerbils (*Meriones unguiculatus* - Ågren et al. 1989), and arctic ground squirrels (*Spermophilus parryii* - Zazula et al. 2006).

A number of nonfossorial mammals dig burrows and tunnel systems. These subterranean excavations serve a variety of functions, including protection from predators, food storage, shelter from environmental elements, and nesting (Reichman and Smith 1990). Burrowing adaptations providing suitable microclimate can be more important in the steppes, which are much more exposed to direct climatic influences than forest biotopes (Bethge et al. 2004; McCafferty et al. 2003).

Most burrows contain nest chambers lined with plant material or fur. In general, nests of burrowing small mammals insulate them from fluctuating ambient temperatures (Casey 1981). The insulation quality of nests themselves is dependent on several factors, such as nest structure (McGowan et al. 2004; Redman et al. 1999), size (height, thickness, and volume - Grubbauer and Hoi 1996; Redman et al. 1999; Szentirmai et al. 2005), nest material (Mertens 1977), and its moisture content. Moisturizing the nest material of ground squirrels reduced insulation quality (Gedeon et al. 2010; McGowan et al. 2004), while building the nest deep in the soil further improves its insulating capacities. Communal nesting further enhances the thermoregulatory function of the nests.

Overwintering in family groups can function as group thermoregulation, which is widespread in mammals. Huddling at cold temperatures is known in deer mice (*Peromyscus*

spp. - Howard 1951), Taiga vole (*Microtus xanthognathus* - Wolff and Lidicker 1981), rats (*Rattus norvegicus* - Alberts 1978), and rabbits (*Oryctolagus cuniculus* - Bautista et al. 2003).

Mound-building mice (*Mus spicilegus* Petényi 1882) inhabit arable lands where they construct large mounds in autumn in which seeds, spikes, and other kinds of plant materials are cached. Underneath the mound the mice excavate a set of galleries with one or two nest chambers and spend the winter there (Murariu 1981; Naumov 1940; Sokolov et al. 1998). Plant material is believed to provide food when it is not available outside the mound (Sokolov et al. 1998). Previous studies found that these mounds vary in size among geographical locations (Bihari 2004b; Hölzl et al. 2009), and the cached plant material represents only a few of the available species (Bihari 2004b; Hölzl et al. 2009). After the winter the remaining mounds contain decayed but consumable plant material; consequently, food storage seems to be a partial explanation for mound building. The mound also could provide shelter against harsh environmental conditions or predators (Bihari 2004b).

The number of animals per mound varies greatly. Researchers used different techniques, from trapping to digging, to measure the number of inhabitants. Data varied from 11.6 ± 2.4 (mean \pm SE) with a male-biased sex ratio of 1.7 (Gouat et al. 2003a) to 1 to 21 with a predominance of males (57.2% - Canady et al. 2009), 1 to 11 (Hölzl et al. 2009), 11.4 ± 2.4 (mean \pm SE) with a male-biased sex ratio of 1.59 (Poteaux et al. 2008), 1 to 11 with an 1.06 sex ratio (Garza et al. 1997), 5–14 (Muntyanu 1990), and 1–14 individuals (Sokolov et al. 1998) per mound. Individuals in a mound are mostly juveniles born in late summer and early autumn. With the exception of rare adult females, adult mice are not found inside mounds and disappear soon after their construction (Canady et al. 2009; Garza et al. 1997; Milishnikov et al. 1998; Poteaux et al. 2008; Simeonovska-Nikolova 2007). Individuals inhabiting the mound can belong to a single litter but more commonly are from related female parents and their unrelated mates (Garza et al. 1997).

Mound-building mice display a high level of seasonality in reproduction. Overwintering individuals delay their sexual maturation until early spring when they leave the mound, disperse, and reproduce (Milishnikov et al. 1998; Naumov 1940; Orsini et al. 1983; Simeonovska-Nikolova and Gerasimov 2000). The delay in reproduction observed in

autumnal individuals is the result of the social effects of living in groups as opposed to the environmental conditions of winter (Feron and Gheusi 2003; Gouat et al. 2003a).

The mound-building mouse is believed to be the only *Mus* species to exhibit a mating system of social monogamy with stable male–female associations (Baudoin et al. 2005; Patris et al. 2002). Studies of captive mound-building mice under laboratory and seminatural conditions showed that they display behavioral features characteristic of social monogamy: establishment of strong social bonds between females and their familiar male (Patris and Baudoin 1998), high aggressiveness of female and male mice towards unfamiliar individuals of both sexes (Patris et al. 2002; Simeonovska-Nikolova 2003; Suchomelova et al. 1998), formation of stable male–female associations in experimental groups (Baudoin et al. 2005; Simeonovska-Nikolova 2003), low reproductive success of the polygynous females (Gouat and Feron 2005), and cooperation between males and females in parental care of their young (Patris and Baudoin 2000). Simeonovska-Nikolova and Gerasimov (2000) found that in northern Bulgaria female mice had exclusive home ranges during the reproductive period. The most frequent spatial association in an agricultural field in north Bulgaria during the reproductive period was between a single male and a female, but other associations between two or three males or one male and two or three females also were observed; female social groups seldom were observed (Simeonovska-Nikolova 2007). Field data are insufficient to confirm that the mating system of these mice is monogamous, and investigation of spatial association in an agricultural field in Hungary demonstrated that polygyny also could occur, at least during the beginning of the reproductive period under conditions of female-biased sex ratio and high population density (Gouat et al. 2003b).

The goal of our study was to describe variation in mound morphology in different soil types and to test two different explanations for mound building: (1) the food-caching function by comparing the composition of the cached plant material in mounds of wild mice to the actual diet, and (2) the heat and water insulation capacity of mounds by measuring temperature and water content of soil beneath mounds and at random points. We expected that if mounds function as food storage, the composition of fecal pellets will be similar to the stored plant composition. We predicted that large mounds can serve as insulators because of both the increased earth layer between the cold winter air and the nest chamber

and the cached plant filling acting as a sponge, which absorbs precipitation thus keeping the soil beneath it drier.

MATERIALS AND METHODS

To describe variations in mound morphology we measured and excavated mounds in 10 locations in Hungary between 8 November 2007 and 8 March 2008 (Table II/1-1.). In all fields crops were harvested at least two months prior to the investigations or where no signs of agricultural activity were evident (fields were abandoned). The climate of Hungary is strongly continental. Precipitation in the autumn of 2007 in all locations was between 110 mm (Sződ) and 210 mm (Vizsoly). Average temperatures ranged from 7.7 °C (Vizsoly) to 9.7 °C (Tiszapüspöki). Examined mounds numbered 7-13 per location. To evaluate the effect of the soil type on mound morphology we chose 5 sandy soil and 5 clay soil locations based on the AGROTOPO soil distribution map of Hungary (Pásztor et al. 1996) compiled by the Research Institute of Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences. At some of these locations mounds constructed in the previous years also were found, but only mounds constructed during the autumn of 2007 were sampled.

Overwintering of mice colonies was followed by excavation of the full burrow systems from October to March, which is a critical period in the survival of colonies of many mice species. Morphological properties of mounds (height, length, width) were measured prior to any other data collection. The volume of the mounds was calculated using the formula for

ellipsoid-base cones $\left(\frac{1}{3}r_1r_2h\pi\right)$, where r_1 is the longer radius, r_2 is the shorter radius, h is the height. The plant volume and plant fill ratio in mounds were measured by destroying the mounds after data collection. Volumes are reported in dm³ unless stated otherwise. Plant fill ratios are calculated as plant volume / mound volume.

We collected samples of mound fills to determine plant species composition in two locations (Kápolna on 6 March 2008 and Sződ on 8 March 2008). Mounds were treated as sample units. From each mound fill ten subsamples (equaling about one-third of the entire material) were taken randomly and sorted by species. Dry weight of each plant species from each sample was measured to the nearest 0.1 g. Average proportion of plant species of the total biomass of sampled mound fill material was calculated over 10 subsamples. To identify

which plants were available for the mice, all plants were harvested from 1 m x 1-m sampling quadrats from a 1-m distance from the edge of each mound and the plant composition was evaluated. Because nests contained exclusively leaves, only monocot-dicot composition was determined. The plant species presented in the study area were collected, and reference microscope slides were prepared for comparative purposes. Special effort was made to collect different parts (stems, flowers, fruits, stolons, etc.) from each of the plants.

Diet composition was evaluated with the technique of microhistological analysis of feces (Mátrai et al. 1998). Pellets were collected from every individual at time of capture. From each pellet 100 remnants of plant epidermis remains were identified to calculate the relative ratio of that species in the diet (Katona and Altbäcker 2002). Each pellet was analyzed individually and then average composition of pellets from individuals belonging to the same mound were used (13 mounds with an average of 6.4 ± 1.7 captured mice per mound at Kápolna and 9 mounds with an average of 6.1 ± 1.4 captured mice per mound at Sződ).

Measurement of thermal and water insulation of mounds, along with other data collection, took place in two geographically distant regions in Hungary (near Tiszapüspöki and Bugyi settlements). All examined mounds were built in the preceding autumn.

To test the thermal insulation of the mounds at Tiszapüspöki we measured temperature differences between the surface and the bottom of 10 mounds (average height = 30 cm). We used submersible laboratory thermometers (TFA Lab Thermometer LT-102, Dostmann GmbH, Wertheim-Reicholzheim, Germany) attached to the end of rods. One sensor was pressed 5 cm below the surface of the mound, and another was placed on the original soil surface beneath the center. For comparison, temperature also was measured in 10 reference locations at 5 cm below the surface and at the depth representing the height of the mound. Each reference location was placed 2 m from the paired mound. To avoid the warming effect of friction while inserting the sensors, all data were taken 20 min after positioning the thermometers, and several sensors were used simultaneously.

To measure the water insulation property of mounds we took approximately 300 g of soil samples from 30 cm under each of the 10 mounds and from the same depths at the 10 reference locations. The samples were taken in December after 2 weeks of rainy weather.

Samples were stored in hermetically sealed plastic bags until they were processed. Water content of the soil samples was calculated from the weight difference measured on a digital scale before and after the sample was dried in an oven at 200 °C until weight remained constant.

For evaluating the insulation effect of the mounds at the nest chamber, more detailed measurements were carried out in arable fields of Bugyi settlement, where soil temperatures were measured at three levels. After determining the average depth of the nest chamber in this location (average depth = 81.4 cm), while digging them out and capturing the mice we randomly chose 10 intact mounds of approximately equal height (30 cm) and measured the temperature of each with submersible laboratory thermometers attached to the end of rods at the following three depths: 5 cm below the surface of the mound, under the mound (original soil surface, or mound depth), and at nest depth (~80 cm below original soil surface). We compared these to data taken parallel from random locations (reference points) 2 m from the mounds at three depths: 5 cm below the soil surface, 30 cm below the surface (mound depth); and at nest depth (~80 cm below earth surface).

Instead of trapping mice, mounds were excavated in a special way to collect all of its inhabitants. First, an 80 cm deep ditch was dug around the mound in a 1.5 m radius because all of the burrow entrance holes belonging to the mound were within this distance. Then the soil was removed gradually from the periphery, and each mouse was caught. Finally, we carefully removed and stored the nest. Animals attempting to escape were unable to leave the basin, thus the number of captured mice was considered the size of the colony. We considered this method necessary because during earlier attempts at capture mice left the burrows through far entrances and even entered the tunnels of neighboring mounds while being chased. All ditches were filled data collection was completed, and the animals were transported to the Biological Research Station at Eötvös University in individual labeled boxes, where fecal pellets were collected. The pellets collected were stored in 70% ethanol until analysis.

TABLE II/1-1.

SITES USED TO SAMPLE AND EXCAVATE MOUNDS OF MUS SPICILEGUS FOR MOUND MORPHOLOGY AND COLLECTION OF MICE.

Settlement (site)	Soil type	Coordinates	Field type	Sample size
Aszód	Clay	47° 39.45'N, 19° 36.56'E	harvested corn	10 mounds
Vizsoly	Clay	48° 23.78'N, 21° 11.32'E	abandoned	10 mounds
Dávod	Clay	45° 58.35'N, 18° 55.08'E	harvested corn	10 mounds
Dunakeszi	Sand	47° 39.27'N, 19° 8.43'E	abandoned	7 mounds
Bugyi	Sand	47° 14.79'N, 19° 9.90'E	abandoned	8 mounds
Tiszapüspöki	Clay	47° 11.86'N, 20° 20.72'E	pasture	10 mounds
Fót	Sand	47° 42.53'N, 19° 10.61'E	abandoned	8 mounds
Rákospalota	Sand	47° 33.25'N, 19° 9.33'E	harvested alfalfa	8 mounds
Kápolna	Clay	47° 46.34'N, 20° 16.65'E	harvested corn	13 mounds
Szód	Sand	47° 43.80'N, 19° 9.85'E	abandoned	9 mounds



FIGURE II/1-1. DIGGING A DITCH AROUND A MOUND TO PREVENT FLEEING OF THE ANIMALS (PHOTO BY THE AUTHOR; NOT PART OF THE ORIGINAL PUBLICATION).

ANIMAL TREATMENT

The procedure used to handle mammals was approved by the Ethical Committee for Animal Experiments at Eötvös University, and conformed to the guidelines of the American Society of Mammalogists (Gannon and Sikes 2007) and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

DATA ANALYSIS

Data were analyzed using Statistica version 8.0 statistical software (StatSoft Inc. 2007). Variables were tested for normal distribution with the Kolmogorov-Smirnov test and Levene's test was used to analyze homogeneity of variances. Data on mound morphology between sampling sites and data on plant use were compared by ANOVA. Tukey's post-hoc test was used to mark significant differences. Data for mound morphology between different soil types was compared using t-tests with Welch's correction. Averages for locations were used as sample units. Temperature and moisture content of mounds and nearest random points were compared using paired t-tests or mixed-effect models with temperatures at different depths as within-subject factors and mound type (mound or reference point) as a categorical predictor. Identification of pairs was used as a covariate. Spearman rank-order correlation (r_s) was used to evaluate the relationship between the soil's water content, or mouse colony size, and mound features. Change in the number of mound inhabitants during winter was analyzed with linear regression. Summary data are reported as mean + *SD*, unless stated otherwise. In all tests a significant statistical difference was assumed when $P < 0.05$.

RESULTS

We found a positive correlation between the number of mice inhabiting the mound and the mound volume ($r_s = 0.314$, $n = 72$, $P < 0.01$), the plant fill volume ($r_s = 0.386$, $n = 43$, $P < 0.01$), and the plant fill ratio ($r_s = 0.339$, $n = 43$, $P < 0.05$). Although mound volume differed among sites ($F_{9,83} = 8.12$, $P < 0.0001$), the volume of mounds grouped by soil type (sand versus clay) did not differ. Mounds differed by soil type in two variables; nests were dug twice as deep in sandy soil, and these mounds contained more plants than mounds constructed on clay ([Table II/1-2](#)).

TABLE II/1-2

CHARACTERISTICS OF MOUNDS CONSTRUCTED BY MUS SPICILEGUS ON HARD (CLAY) AND SOFT (SANDY) SOIL. AVERAGES OF SAMPLING SITES WERE TREATED AS SAMPLE UNITS. WELCH'S CORRECTED T-TEST WAS USED TO COMPARE DATA.

Mound feature	Total <i>n</i> = 10 mean	<i>SD</i>	Clay <i>n</i> = 5 mean	<i>SD</i>	Sandy <i>n</i> = 5 mean	<i>SD</i>	<i>t</i>	<i>P</i>
Height (cm)	27.3	4.2	28.8	1.5	25.7	5.6	1.17	0.31
Length (cm)	147.4	23.1	146.0	15.8	148.7	3	0.17	0.87
Width (cm)	119.8	21.2	119.1	9.4	120.4	30.4	0.08	0.93
Volume (dm ³)	139.2	63.6	137.4	23.2	141.0	92.4	0.08	0.94
Plant fill (dm ³)	38.0	10.1	29.6	3.6	46.4	5.9	5.42	<0.01
Plant fill ratio (plant vol/ mound vol)	0.27	0.04	0.26	0.06	0.28	0.02	0.94	0.38
Nest depth (cm)	61.6	25.6	39.1	9.7	84.3	10.3	7.13	<0.001
Mice per mound	6.6	1.1	6.7	1.3	6.4	0.6	0.47	0.66
Sex ratio (male/female)	0.53	0.06	0.52	0.04	0.54	0.06	0.58	0.57

Composition of available plants at the two examined locations was slightly different. Most common plants at Kápolna by weight were *Echinochloa* sp. (75.8%), *Zea mays* (17.6%), and *Setaria* spp. (2.33%). Most common plants at Szöd were *Chenopodium* spp. (53.9%), *Setaria* spp. (25.9%), *Ambrosia* sp. (14.1%), and *Echinochloa* sp. (5.2%). Plant use was site- and function-dependent (Kápolna: $F_{3,27} = 107.06$, $P < 0.0001$; Szöd: $F_{3,19} = 103.56$, $P < 0.0001$). At both sites nests were constructed from monocots, but mice fed mostly on dicots (Figure II/1-2).

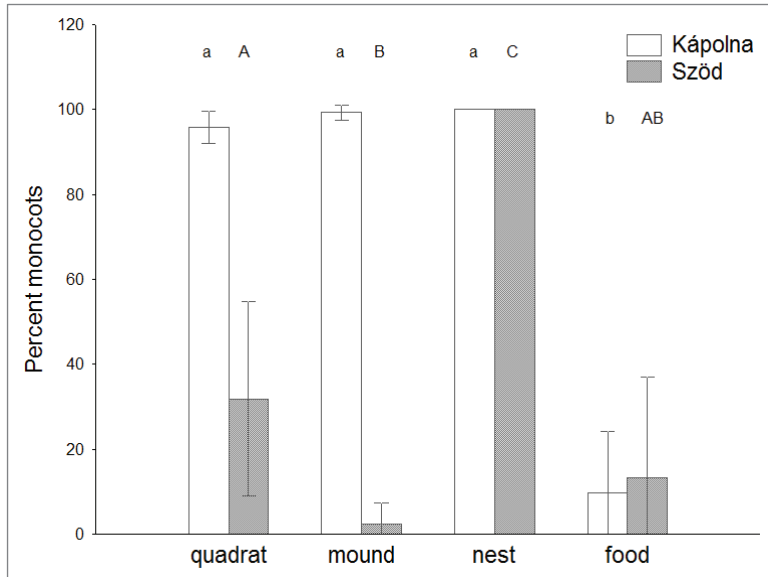


FIGURE II/1-2 MONOCOT PLANT USE BY MUS SPICILEGUS AT TWO LOCATIONS. COLUMNS PRESENT MEAN (\pm SD) PERCENT BY WEIGHT (SAMPLING QUADRAT, MOUND, NEST) AND MEAN (\pm SD) PERCENT OF REMNANTS FROM FECES (FOOD) THAT WERE MONOCOTS. DIFFERENT LETTERS ABOVE THE COLUMNS INDICATE SIGNIFICANT DIFFERENCES ($P < 0.05$) WITHIN KÁPOLNA (LOWER-CASE LETTERS) AND SZÖD (UPPER-CASE LETTERS) LOCATIONS REVEALED BY A POST HOC TUKEY TEST.

Mound fill material was site-specific; it contained mostly only one species, *Echinochloa* sp. at Kápolna and *Chenopodium* sp. at Szöd. The diet of the mice was more diverse but contained only a few plant species surrounding the mounds. The plants most often consumed were *Amaranthus* sp., *Ambrosia* sp., and *Dactylis* sp. Overlap between plants eaten and mound fill composition was extremely low, and even when a small overlap appeared on average, the actual mound and dweller diet composition was different, thus excluding the possibility that stored plants were eaten (Figure II/1-3).

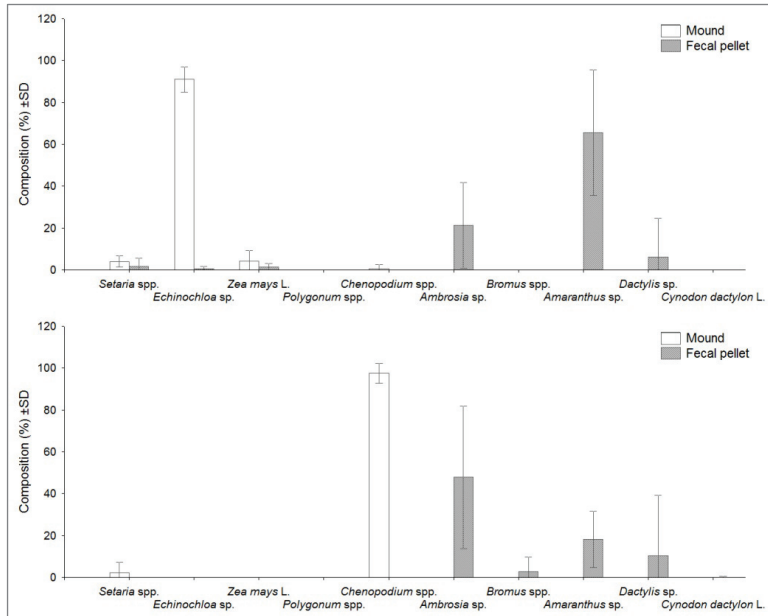


FIGURE II/1-3 PLANT COMPOSITION BY SPECIES OF MOUNDS AND FECAL PELLETS OF *MUS SPICILEGUS* AT A) KÁPOLNA AND B) SZÖD. COLUMNS PRESENT MEAN (\pm SD) PERCENT OF SPECIES BY WEIGHT (MOUNDS) AND MEAN (\pm SD) PERCENT OF REMNANTS FROM FECES IDENTIFIED AS PARTICULAR SPECIES (FECAL PELLET).

At the site near Tiszapüspöki, after a cold night in early winter, morning surface temperature was colder on mice mound tops than on paired reference points ($t_9 = 3.21$, $P < 0.01$). Temperatures under the mounds were higher than at paired reference points ($t_9 = 2.98$, $P < 0.01$). Temperature differences between the surface and the soil show that mice mounds have better insulating property than soil at equal height ($t_9 = 3.62$, $P < 0.01$; [Figure II/1-4](#)).

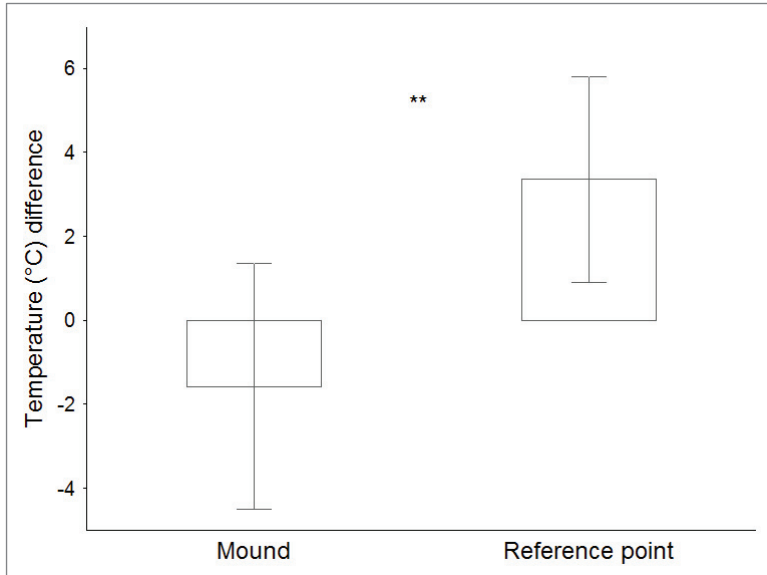


FIGURE II/1-4 MEAN (+ SD) TEMPERATURE DIFFERENCE BETWEEN THE SURFACE AND THE BASE OF MOUNDS OF *MUS SPICILEGUS* AND BETWEEN THE SURFACE AND THE SOIL AT -30 CM (REFERENCE POINT) AT THE SITE NEAR TISZAPÜSPÖKI.

The water content of the soil was lower under the mounds than in random points at the same depth ($t_9 = 4.00$, $P < 0.01$; [Figure II/1-5](#)).

Soil water content under the mounds correlated negatively with the height ($r_s = -0.656$, $n = 10$, $P < 0.05$), volume ($r_s = -0.696$, $n = 10$, $P < 0.05$), and the plant fill ratio of mounds ($r_s = -0.841$, $n = 10$, $P < 0.001$). At the site near Bugyi, where temperature was measured at the depth of the nest as well, the surface of the mounds were slightly cooler, and the temperature at the nest depth was warmer under the mounds ($F_{6,32} = 40.39$, $P < 0.001$; [Figure II/1-6](#)).

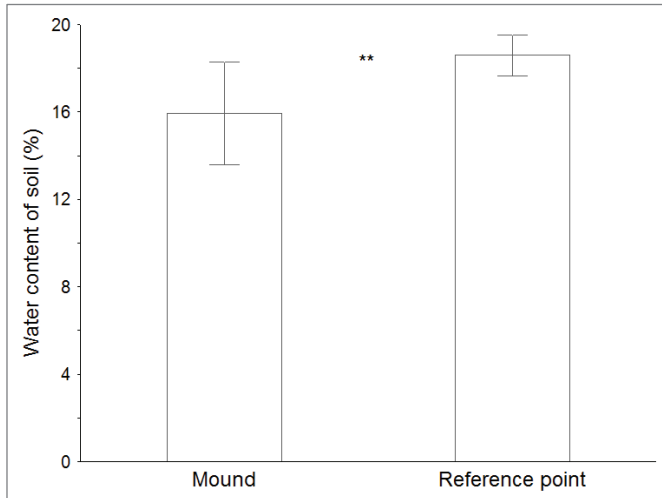


FIGURE II/1-5 MEAN (+ SD) PERCENT WATER CONTENT OF THE SOIL AT 30 CM DEPTH BENEATH THE MOUNDS OF *MUS SPICILEGUS* AND IN THE SAME DEPTH BENEATH THE REFERENCE POINTS AT THE SITE NEAR TISZAPÜSPÖKI.

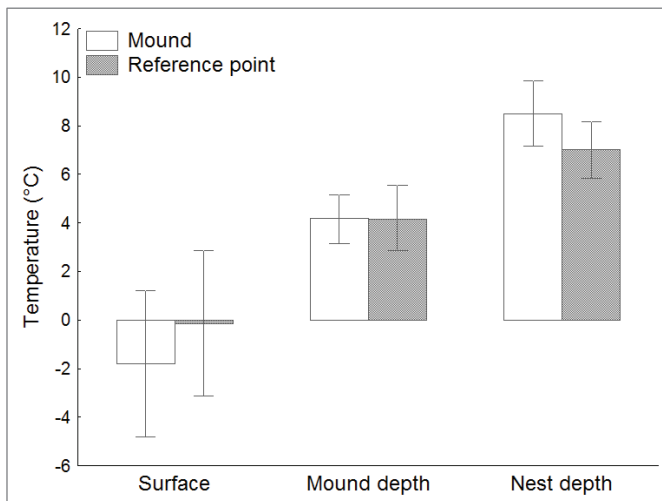


FIGURE II/1-6 MEAN (+ SD) TEMPERATURE AT DIFFERENT LEVELS IN THE MOUNDS OF *MUS SPICILEGUS* AND AT THE REFERENCE POINTS.

Mouse colony size showed no decrease during winter in either soil type (sand: $F_{1,29} = 0.763$ $P = 0.39$; clay $F_{1,39} = 0.151$, $P = 0.34$; [Figure II/1-7](#)). This indicated successful overwintering in this species.

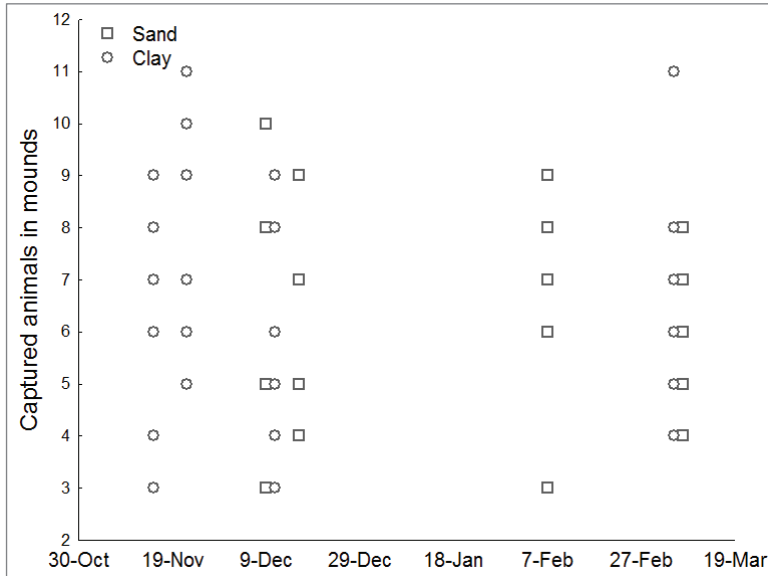


FIGURE II/1-7 NUMBER OF *MUS SPICILEGUS* CAPTURED ON CLAY AND SANDY SOIL THROUGH THE WINTER. DATA POINTS REPRESENT THE MOUNDS.

DISCUSSION

Mounds of mound-building mice, *Mus spicilegus*, were large constructions, their total volume reaching almost 300 l. We found mound sizes consistent with reports of other studies (Hözl et al. 2009; Pisareva 1948; Sokolov et al. 1990); however, mound sizes were less variable than those found by Hözl et al. (2009). Furthermore, mound size and plant fill volume correlated positively with the number of mice, as previously found by Sokolov et al. (1990), but this contradicts the findings of Hözl et al. (2009). Results among studies could have differed because of different methods scientist used to evaluate the number of mice inhabiting the mounds. During our earlier attempts we noticed that mice left the burrows

through distant entrances (up to 1 m from the mound periphery) when we dug near the nest chamber and even entered the tunnels of neighboring mounds while being chased. To obtain the most accurate number of mice without confusing individuals we dug a trench around the mound so that all burrow entrances belonging to the mound were within this distance. Then the soil was removed gradually from the periphery until all tunnels were opened. We believe that this method is unique so all comparisons with other studies should be made with caution.

By comparing the characteristics of mounds on soft sandy soil and hard clay, we expected that the morphology of mounds would reflect the physical characteristics of the soils. The size of the mounds varied among sampling sites, but it was independent of soil type, except that mice dug their nest deeper in soft soil.

Mounds contain several dozen liters of plant material covered by a soil layer, which is believed to provide food when other plants are not available (Sokolov et al. 1998). By comparing the composition of the cached plant material in mounds to the actual diet of mice, we did not find any supporting evidence for the common belief that mounds serve as winter food caches in this species. Based on their fecal pellet composition, mice consumed mostly dicots, which also exclude the possibility that the nest material made exclusively from grass was used as food. Winter food supply is key to their survival, and mound-building mice seemed to obtain food from the vegetation surrounding the mound and not from the mound fill itself. A reduced likelihood of mound fill being used as a food source also was suggested by the absence of tunnels from the nest to that part of the mound. Dicots used either as food or as mound fill material were common weeds associated with initial successional stages on abandoned land. However, our results do not preclude the possibility of a food storage function of the mound later in winter or in early autumn when available food around the mounds is scarce.

We expected that mounds function as insulators and found a slightly elevated soil temperature under the mounds, where the soil was also dryer than in neighboring random points. Mound quality (volume and plant fill ratio) and water-insulating capacity correlated positively, suggesting that mound condition is a good indicator of colony success.

Overwintering of mice colonies was successful, as colony size was reasonably stable throughout the winter.

Nests of mound-building mice were about 60-80 cm from the newly established soil surface, even deeper in soft sandy soils, and we found elevated soil temperatures under the mounds. We also found that soil was drier under the mounds, indicating that insulation capacity of mounds against water adds an extra protection to thermal advantages, although we do not know how much these factors contribute to insulation. Both the thick grass nest, the plant fill of the mound, and the soil itself might have contributed to the reduced temperature fluctuations. As various measures of mound size correlated positively with both the water-insulating capacity and mouse colony size, an insulation function for mounds is plausible. However, a manipulative study testing the significance of variation in mound size would provide additional clarification.

The mound as a large heap of soil might provide extra protection not only against the harsh winter conditions but also against ground predators attempting to reach the mice. While visiting fields where mound-building mice live, we noticed that only a small fraction of mounds were visited and only partially opened by ground predators, but further investigations are needed to determine if mounds provide protection from ground predators.

Nest and mound building in autumn increases the probability of overwintering and is critical for small mammals in temperate and subarctic climates (Banks and Dickman 2000; Natori and Porter 2007; Pitt et al. 2008) when even small energy savings can influence survival (Geiser 1998; Körtner and Geiser 2000; Lovegrove et al. 2001; Németh et al. 2009). Winter nests provide insulation from fluctuating ambient temperatures (Casey 1981), and mounds of mound-building mice also reduced temperature changes and resulted in an elevated soil temperature at nest depth. As a result, mounds can improve energy conservation and thereby increase winter survival (Bethge et al. 2004; McCafferty et al. 2003; Redman et al. 1999).

Energy conservation during the winter also could contribute to better breeding success (Lamprecht and Schmolz 2004) when mice leave the mound to breed in the next spring (Millesi et al. 2002). The present study shows that constructing communal plant caches

covered by soil for insulating against cold and water, combined with hiding in a well-constructed nest beneath the frost, seem to be a successful overwintering strategy for mound-building mice in regions with a temperate climate.

ACKNOWLEDGMENTS

This work was supported by the GEBACO EU FP6 Grant no. 28696 to V. Altbäcker. We thank the collaborating colleagues of the Ethology Department, especially Károly Csizmadia and András Kosztolányi, for their contribution to gathering and analyzing the data. We thank Patricia Dévényi for suggestions regarding the manuscript.

II/2. THE CONTRIBUTION OF THE VEGETABLE MATERIAL LAYER TO THE INSULATION
CAPACITIES AND WATER PROOFING OF ARTIFICIAL *MUS SPICILEGUS* MOUNDS

Péter Szenczi*, Dániel Kopcsó, Oxána Bánszegi, Vilmos Altbäcker

Published in: Mammalian Biology - Zeitschrift für Säugetierkunde

Volume 77, Issue 5, September 2012, Pages 327–331

ABSTRACT

Successful overwintering of small mammals in temperate and cold climates requires behavioral and physiological adaptations. There are several strategies to survive food shortages and the cold. Most species of small mammals use multiple methods simultaneously but nest building and burrowing are the most widespread among them. A well-constructed, dry nest insulates animals from harsh ambient conditions. Mound-building mice build large banks in the autumn and establish a burrow system with nest chambers beneath them. These overwintering structures are built from soil and a considerable amount of plant material. Recent studies presume that the stored vegetable matter is not, or not exclusively serve as food and indicate that the mounds might have insulating role. To investigate the function of their plant fill, we have built artificial mounds with varying plant content, similar to those built by mound-building mice. We have measured temperature change at three levels, at the surface, under the mound and at the nest depth, and investigated their water retaining properties. We showed that the plant fill plays a major role in their thermal insulation and waterproofing properties. Mounds reduced temperature variation of the soil and may protect the nest from absorbing precipitation during the winter.

Keywords: mound-building mouse, overwintering, insulation, burrowing, nest quality

INTRODUCTION

Mammals in temperate and cold climates develop several physiological and behavioral adaptations to increase heat production or reduce heat loss during winter. However, it is still generally observed that this is the time period when small mammals experience their greatest mortality. Large population declines during the winter have been noted in bank voles *Clethrionomys glareolus* (Gipps et al. 1985; Pucek et al. 1993), short-tailed field voles *Microtus agrestis* (Bradshaw 1992), tundra voles *Microtus oeconomus* (Aars and Ims 2002) yellow-necked mice *Apodemus flavicollis* (Pucek et al. 1993) and house mice *Mus musculus* (Berry et al. 1973). Hence, traits that help survival in harsh winter conditions are of special importance.

Burrowing and nest building along with huddling are wide-spread behavioral adaptation that may be responsible for increased tolerance of low temperatures (Reichman and Smith 1990). Most burrows contain nest chambers lined with plant material or fur. In general, their main role is to insulate animals from ambient temperatures (Casey 1981) and may also serve as protection from predators or as food storage (Reichman and Smith 1990). The energy conservation that can be achieved through nest building is considered crucial (Bethge et al. 2004; Geiser 1988; Houston and McNamara 1993; Lovegrove et al. 2001; McCafferty et al. 2003; Redman et al. 1999) or even critical for winter survival (Barclay et al. 2001; Pinowski et al. 2006). Building the nest deeper in the soil further improves its insulating properties. Clay soils typically have higher heat capacities than sandy soils, and increasing the organic matter content typically increases moisture retention and heat capacity.

The insulation quality of the nests of small mammals and birds depend on several factors, such as nest structure (McGowan et al. 2004; Redman et al. 1999), size, height, thickness, volume (Grubbauer and Hoi 1996; Redman et al. 1999; Szentirmai et al. 2005), nest material (Mertens 1977), and moisture content (Hilton et al. 2004; Pinowski et al. 2006). Laboratory studies confirm that moistening the nest material of ground squirrels considerably reduced the quality of insulation (Gedeon et al. 2010). A flooded or wet nest can be fatal for the occupants due to the increase in thermal conductivity.

Mound-building mice (*Mus spicilegus* Petényi 1882, as MBM hereafter) construct large mounds during autumn in which seeds and other plant materials are cached. The mounds are built by hoarding seeds and ears to a pile first and then covering it with soil resulting in a round or oval based heap, with plant material at the bottom and soil on the surface. The mice excavate one or two nest chambers underneath the mound to spend the winter there (Murariu 1981; Naumov 1940; Sokolov et al. 1998). Mice build their nests at varying depths - between 30 cm and 80 cm - depending on the type of soil (Canady et al. 2009; Szenczi et al. 2011). The cached plant material represents about 1/3 of the mound volume. Its composition is determined by the characteristics of the habitat, but contains only a few of the available species. The mice preferentially use certain plants usually those which can be found in big quantities in the vicinity, but preference for mound building and food not necessarily match (Bihari 2004b; Hölzl et al. 2009; Hölzl et al. 2011b; Szenczi et al. 2011).

Current studies question the former concept that the piled seeds in the mound serve exclusively as food as the thermal insulating and waterproofing properties of mounds were demonstrated along with the lack of evidence of consumption during winter. Hölzl et al. (2011a) revealed that the size of mounds influence their thermal insulating capacities. In field studies Szenczi et al. (2011) found that the soil beneath the mounds is drier and mounds have better insulating property than soil at equal height, while could not trace remnants of the collected seeds in the mice pellets. Sometimes non-edible parts of plant are being collected (e.g. mound built almost entirely of chamomile flowers) or mice use less nourishing plants, like pig-weed in a corn-field (Hölzl et al. 2009). In a recent study Serra et al. (2012) demonstrated that mound building behavior can be triggered under autumn conditions by offering cotton balls as building materials. Collecting plant material for mound fill is surely more time consuming than building the mound entirely of dirt. If it is not for food the plant material layer should have other functions.

In this study we attempted to determine the role of plant fill in the thermal insulation and waterproofing properties of artificial mounds with varying plant fill ratios. We assumed that a) mounds have a significant effect on temperature and water content of the soil beneath them and b) mounds with higher ratio of plant material perform better in thermal insulation and have reduced water penetration.

MATERIAL AND METHODS

In order to test the role of plant filling of mounds on the overwintering conditions we measured the change of temperature and water content of the soil in and under the artificial mounds. Mounds were built in the garden of the Biological Research Station of Eötvös University at Göd, Hungary (47°40.903'N 19°7.849'E) between 15 February and 28 February 2009. The mean temperature in the region in February is 1.5 °C (second coldest month) with an average monthly precipitation of 36.7 mm (from which 20.2 mm is snow). The size and structure of the mounds and the depth of the assumed nest chambers were based on the average MBM mounds under natural conditions on fields with identical soil characteristics. In our previous study we found that the average total volume of the mounds is $139.2 \text{ dm}^3 \pm 63.6$ (SD) with a height approximately 30 cm (Szenczi et al. 2011), while their size may vary between habitats (Hölzl et al. 2009). All mounds were built using soil from the vicinity of the test area and barley seeds mixed with straw (1:1 volume) as plant filling. Mounds were built with a 120 cm diameter round base and were 30 cm in height, giving a total volume of 110 - 120 dm³. We imitated the structure of the natural mounds as good as possible ([Figure II/2-1](#)). We spread the plant material on the ground first and gently covered it with soil. We adjusted the plant material content of the mounds by building mounds with 2/3, 1/3 and 0 plant volume ratio; in addition we performed the same measurements on temperature and water content at spots with no mounds.



FIGURE II/2-1 PICTURE OF A MOUND OF MOUND-BUILDING MOUSE (TOP LEFT) AND INNER STRUCTURE, CONTAINING ALMOST EXCLUSIVELY CHAMOMILE FLOWERS (*MATRICARIA SP.*) (TOP RIGHT). PICTURE OF AN ARTIFICIAL MOUNDS USED IN THE EXPERIMENT (BOTTOM LEFT) AND ITS INNER STRUCTURE (BOTTOM RIGHT)

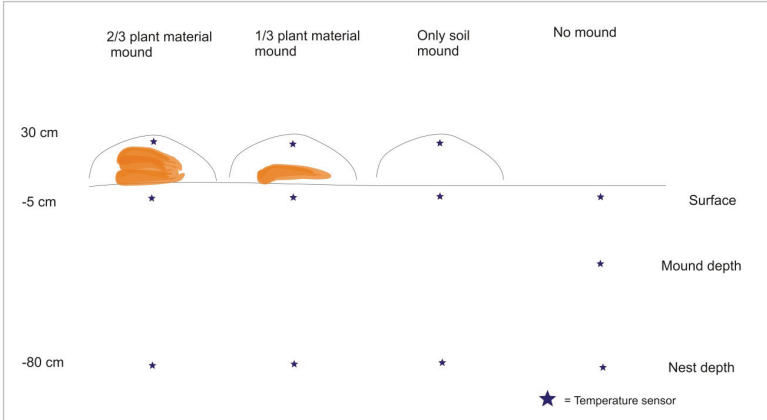


FIGURE II/2-2 POSITION OF THE SENSORS IN THE GROUND (NOT PART OF THE ORIGINAL PUBLICATION)

We used submersible laboratory thermometers (TFA Lab Thermometer LT-102, Dostmann GmbH, Wertheim-Reicholzheim, Germany). The sensors were placed at three different depths. At 5 cm below the surface of the mound, under the mound (original soil surface, or mound depth), and at nest depth (~80 cm below original soil surface). On the 'no mound spots' thermometers were placed 5 cm below the soil surface, 30 cm below the surface (mound depth); and at nest depth (~80 cm below earth surface). Ambient temperature was also measured at 2 meters above ground level. All underground sensors were placed prior to experiment. Mounds were 5 m apart from each other in a 4 x 3 grid. Three repeats of measurements were carried out while different types of mounds were placed randomly in the grid. The building of the mounds began at 8 am and finished by 10 am before each session. The mounds were left undisturbed until the next morning; recording of temperatures began on the next day at 9 am. The thermometers were read every 60 minutes for 24 hours. Daily mean temperatures and daily temperature variations were calculated for each level.

To evaluate the water proofing property of mounds we took 200 g mound fill samples and 200 g soil samples from 60 cm depth before building the mounds to measure their original water content. We chose this depth to make sure not to damage any sensors while digging out the soil samples. After the 24 hours long thermal survey, we slowly poured 11 liters of water on the top of the mounds and reference points. It corresponds to 10 mm of precipitation in reference to the base size of the mounds. A ring shaped cardboard partition was placed around the mounds and reference points to avoid the flowing of water. 120 minutes later we took mound fill and soil samples from the same depth. Samples were stored in hermetically sealed plastic bags until they were processed. Water content of the samples was calculated from the weight difference measured on a digital scale before and after the sample was dried in an oven at 200 °C until weight constancy.

DATA ANALYSIS

Data were analyzed using Statistica version 8.0 statistical software (StatSoft 2007). All data were log transformed to meet conditions of normality for statistical purposes. Mean temperatures and daily temperature variations were compared using general linear models, ambient temperature or ambient temperature variance as continuous predictors. Soil moisture-content change under the mounds was compared by one-way ANOVA. Tukey's

post-hoc tests were used to mark significant differences. Water-content differences in the plant filling were compared using t-test with Welch's correction.

RESULTS

THERMAL INSULATION

Mean temperature on top of the mounds was significantly lower than the mean temperature on the surface without mounds ($F_{3,31} = 25.68$, $P < 0.001$), but there were no differences between mound types. The daily temperature variation was smaller on the soil surface without mounds than with the mounds ($F_{3,31} = 98.26$, $P < 0.001$). (Figure II/2-3)

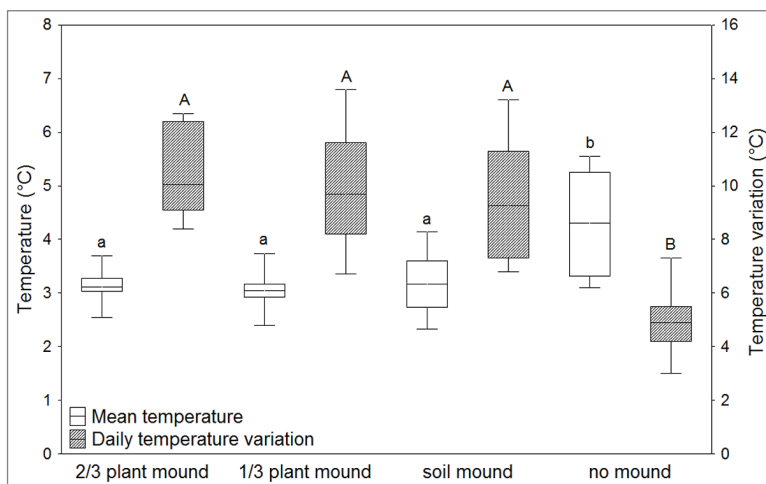


FIGURE II/2-3 MEAN TEMPERATURE AND DAILY TEMPERATURE VARIATION ON THE SURFACE OF DIFFERENT TYPE OF MOUNDS (5 CM BELOW SURFACE). THE DATA ARE PRESENTED AS MEDIAN (THICK LINE), UPPER AND LOWER QUARTILES (BOXES) AND MINIMUM-MAXIMUM (WHISKERS). DIFFERENT LETTERS ABOVE THE BOXES INDICATE SIGNIFICANT DIFFERENCES ($P < 0.05$) IN THE DAILY MEAN TEMPERATURE (LOWERCASE LETTERS) AND DAILY TEMPERATURE VARIATIONS (UPPERCASE LETTERS) REVEALED BY A POST HOC TUKEY TEST.

Measuring at the bottom of mounds and at 30 cm depth at the ‘no mounds’ spots, the daily thermal variation under the plant-containing mounds were lower than under only soil mound and spots without mounds ($F_{3,31} = 17.630$, $P < 0.001$). There were no difference in the mean temperature at the same level ($F_{3,31} = 1.387$, $P = 0.264$). (Figure II/2-4) The layer of plant material with a greater amount of air within was a more efficient insulator over soil effectively subduing the thermal extremes of the surroundings.

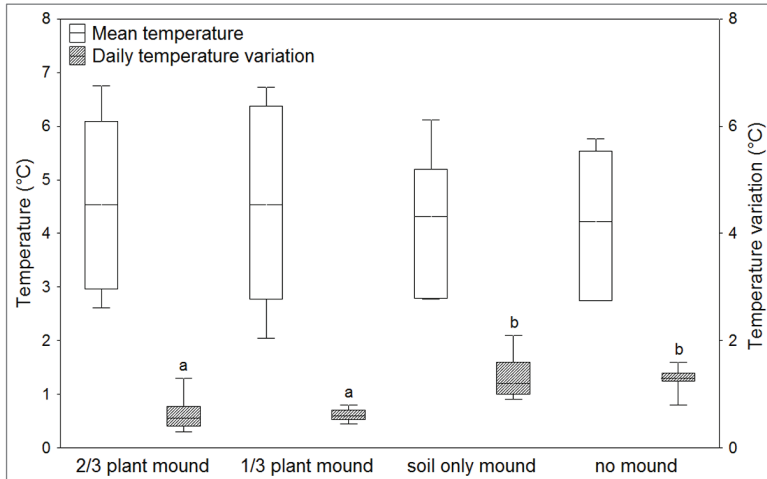


FIGURE II/2-4 MEAN AND DAILY TEMPERATURE VARIATION UNDER DIFFERENT TYPE OF MOUNDS (30 CM BELOW THE SURFACE). THE DATA ARE PRESENTED AS MEDIAN (THICK LINE), UPPER AND LOWER QUARTILES (BOXES) AND MINIMUM–MAXIMUM (WHISKERS). DIFFERENT LETTERS ABOVE THE BOXES INDICATE SIGNIFICANT DIFFERENCES ($P < 0.05$) IN DAILY TEMPERATURE VARIATION REVEALED BY A POST HOC TUKEY TEST.

As expected, mean temperature was higher and temperature variation was lower deeper in the ground. Mean temperature and temperature variation at the nest level was $4.94^{\circ}\text{C} \pm 1.4$ and $0.57^{\circ}\text{C} \pm 0.29$ under 2/3 plant mounds, $4.64^{\circ}\text{C} \pm 1.56$ and $0.47^{\circ}\text{C} \pm 0.14$ under 1/3 plant mounds, $4.83^{\circ}\text{C} \pm 1.57$ and $0.51^{\circ}\text{C} \pm 0.18$ under only soil mounds, $4.45^{\circ}\text{C} \pm 1.3$ and $0.49^{\circ}\text{C} \pm 0.26$ under spots without mounds. We found no difference neither in the mean temperature ($F_{3,31} = 0.892$, $P = 0.455$) nor in the temperature variations ($F_{3,31} = 0.911$, $P =$

0.446) at the level of the nests. In deeper regions the thermal attributes of mounds and insulating properties of plant fills are negligible compared to the effect of the thick dirt layer above and the thermal properties of the surrounding soil.

WATERPROOFING

Mounds seem to be more efficient in reducing water penetration rather than increasing thermal insulation. After irrigating, the water content of the soil elevated significantly at the 'no mound spots' but remained stable under the mounds ($F_{3,31} = 74.861$, $P < 0.0001$). Mounds with plant fill performed better than soil only mounds as the soil was drier after the water treatment than before, probably because the seeds and straw absorbed moisture from the underlying soil (Figure II/2-5) during the time of placement of the mound and measurement.

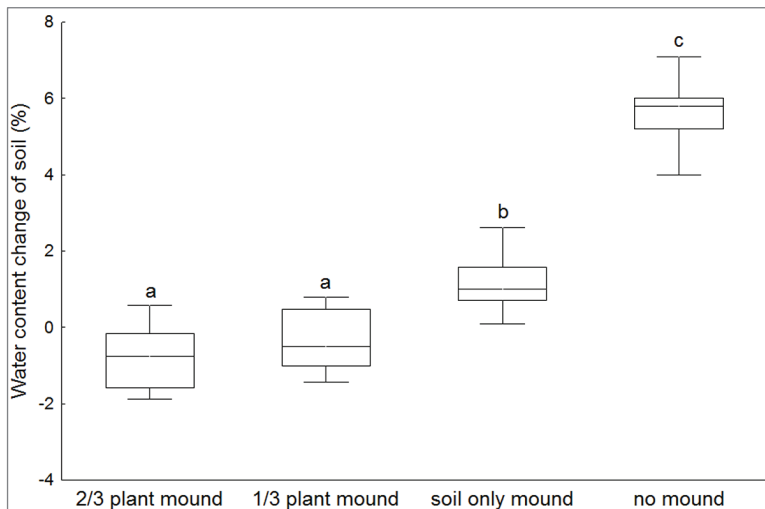


FIGURE II/2-5 CHANGE IN THE WATER CONTENT (%) OF SOIL UNDER DIFFERENT TYPE OF MOUNDS AT 60 CM DEPTH. THE DATA ARE PRESENTED AS MEDIAN (THICK LINE), UPPER AND LOWER QUARTILES (BOXES) AND MINIMUM-MAXIMUM (WHISKERS). DIFFERENT LETTERS ABOVE THE BOXES INDICATE SIGNIFICANT DIFFERENCES ($P < 0.05$) IN DAILY MEAN TEMPERATURE REVEALED BY A POST HOC TUKEY TEST.

As a consequence of their shape, the mounds undoubtedly divert part of the precipitation but the plant fill also has a major role in water proofing. We found that the water content increased in the plant fill of the mounds, and the change was significantly bigger in the 1/3 plant fill ratio mounds ($t_{16} = 2.339$, $P < 0.05$). (Figure II/2-6)

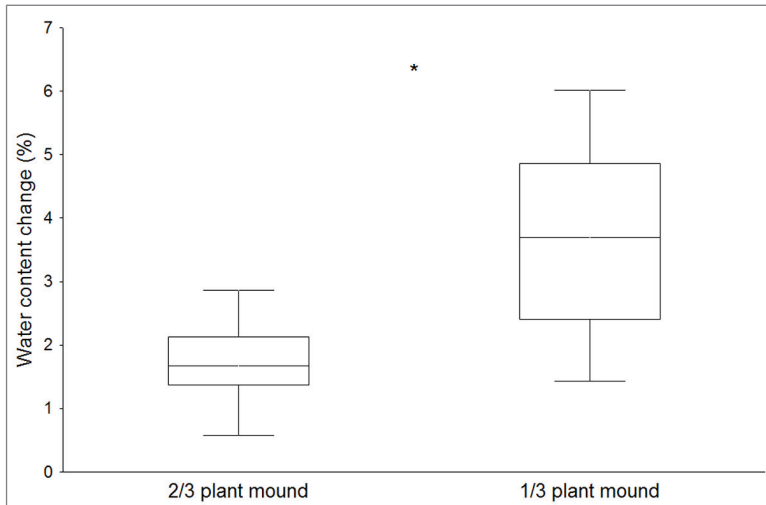


FIGURE II/2-6 CHANGE IN THE WATER CONTENT (%) OF THE PLANT FILL IN DIFFERENT TYPE OF MOUNDS. THE DATA ARE PRESENTED AS MEDIAN (THICK LINE), UPPER AND LOWER QUARTILES (BOXES) AND MINIMUM–MAXIMUM (WHISKERS). ASTERISK MARK SIGNIFICANT DIFFERENCE ($P < 0.05$) REVEALED BY T-TEST WITH WELCH'S CORRECTION.

DISCUSSION

Our results show that mounds influence the thermal characteristics and water content of the soil beneath. The south side of the mounds received solar rays at a steeper angle than normal soil surface, hence heating up more on days with direct sunlight. On the other hand, as protruding objects with greater surface open to cold air they emit heat faster, resulting in lower night temperatures. We found that mounds which contained a plant material layer were better thermal insulators than soil only mounds when measured at the bottom of mounds, as the daily temperature variation was reduced. It is probably the result of the

considerable amount of air, which is trapped in the plant fill, creating a good insulating layer between the ambient temperature and the soil. We were unable to detect significant differences in the daily mean temperature at any level of the different mound types. Furthermore, any thermal effect of the mounds was only detectable at the base of the mounds and on the surface, where the mice spend minimal time since they live in the tunnels and chambers underneath (Festetics 1961; Naumov 1940). Deeper, where the mice build their nests the influence of the surrounding soil was dominant, concealing the effect of the mound on the surface.

According to our results, the water proofing property of the mounds is more evident. When compared the changes in the moisture content of the soil near the hypothetic nest chamber, we found that the soil under the plant containing mounds remained drier. The mound itself probably diverted a significant amount of water through its morphology and the plant material layer worked as an absorbent. In accordance with previous field studies (Szenczi et al. 2011) we found that the mounds of mice are capable of keeping the soil beneath them dry, consequently protecting the nest. Plant containing mounds performed better than only soil mounds, indicating that the vegetable content is important. We were unable to show significant differences between mounds containing 1/3 and 2/3 plant fill. This might be due to the experiment protocol, as adding more water would saturate the smaller plant fill first. In natural mounds the vegetable content usually varies around 1/3. Since collecting seeds and ears is more time consuming over hoarding dirt, this ration could be the result of an optimization process by the mice.

As water content increases thermal conductivity and evaporative heat transfer, moisture content of biological materials is one of the most important variables determining their thermophysical characteristics (Bozikova and Hlavac 2005). Thus, keeping the nest dry during the winter is crucial. A flooded or wet nest is unable to fulfill its role and constructing another one would be extremely energy-consuming or even impossible because of the lack of potential building material. The mounds of the mound-building mice successfully protect the nest from precipitation which may contribute to the winter survival of the inhabitants. While the water content change in this study was not particularly big, the amount of water we used represented only small proportion of the usual precipitation during the season. It

must be considered as well, that an underground nest has limited possibilities of drying out, thus repeated water intakes are likely to be additives.

There are several behavioral adaptations to reduce heat loss and to compensate for the drop of food availability; food hoarding and nest building are the most widespread among them. Establishing a well-protected, good insulating nest or burrow system not only offers protection against predators but defends the inhabitants from the cold and lowers their energy consumption.

Communal overwintering under well-constructed, plant material-filled mounds seems to be a successful strategy of the mound-building mice. Szenczi et al. (2011) were unable to reveal considerable decrease in group size under intact mounds. There is no scientific evidence supporting the food storage function of the mounds but the structure itself undoubtedly has thermal and water proofing properties (Hölzl et al. 2011a; Szenczi et al. 2011).

Animal nests built of plants and fur are highly effective insulators (Casey 1981; Glaser and Lustick 1975), but can only fulfill their task until they remain dry. Constructing the mound from soil and vegetable matter might be more time consuming but the characteristics achieved provide better protection against the harsh winter conditions.

ACKNOWLEDGEMENTS

This work was supported by the GEBACO EU FP6 Grant no. 28696 to V. Altbäcker. We thank the collaborating colleagues of the Ethology Department especially Károly Csizmadia. We thank Patricia Dévényi for suggestions regarding the manuscript.

II/3. NATAL DISPERSAL IN TWO MICE SPECIES WITH CONTRASTING SOCIAL SYSTEMS

Zita Groó*; Péter Szenczi; Oxána Bánszegi; Vilmos Altbäcker

Submitted to: Behavioral Ecology and Sociobiology

ABSTRACT

We compared the natal dispersal behavior of two mice species under laboratory conditions. Natal dispersal is a movement of an animal from its birthplace to its breeding area. This behavior is known to be influenced by the mating system. In polygamous species, males are more likely to disperse, while in most of the monogamous species both sexes disperse. Our subjects, the house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*) are two sympatric species of the genus *Mus*. Both are native in Hungary, but they differ in their habitat type and mating system. The house mouse is a polygynous species and adapted to human environment, known for mature and reproduce early. On the contrary, the mound-building mice is monogamous, and they inhabit abandoned agricultural fields, where they spend the unfavorable winter period in nest chambers under mounds, which they construct from soil and plant material. Successful overwintering for this species demand delayed maturity and reduced dispersion during the winter. Our results showed, that the natal dispersal of these two species differ: both sexes of the mound-building mice dispersed later, than the house mice, where a difference between sexes also occur: house mice males dispersed earlier than females. The mound-building mice showed no sexual dimorphism in this behavior.

Keywords: dispersal, house mouse, mound-building mouse, mating system,

INTRODUCTION

Natal dispersal is the movement of individuals, during which they leave their birthplace and move to different habitats where they will reproduce (Endler 1977; Howard 1960). It is one of the basic life-history traits of a population (Clobert et al. 2004; Dingle 1996; Gadgil 1971) as it influences its genetic structure, and plays a fundamental role in demographic processes (Gaines and McClenaghan 1980). While having its own costs and benefits (Johnson and Gaines 1990; Stenseth and Lidicker 1992), it is under ecological, social and physiological influence (Dufty and Belthoff 2001).

Many vertebrates show sex-biased dispersal, which pattern varies among taxa. Mating systems of species or populations supposed to have a fundamental role in this asymmetry (Dieckmann et al. 1998). There are several hypotheses explaining the role of mating systems in natal dispersal: competition for resources (Greenwood 1980; Greenwood and Harvey 1982), competition for mates (Dobson 1982), and avoidance of inbreeding (Wolff 1993, 1994). As a general rule polygynous/promiscuous species usually display a male-biased dispersal, while both sexes disperse or dispersal is female-biased in monogamous species (Dobson 1982; Favre et al. 1997; Greenwood 1980; Perrin and Mazalov 2000; Wolff 1993).

Natal dispersal usually occurs before reproduction; therefore, its timing may depend on the onset of maturation of an individual (Howard 1960). As social factors have their effect on timing of puberty (Drickamer 1989) their direct impact on dispersion is also important; aggression of the parents towards sexually mature offspring, or the aggression of dominant individuals toward their siblings might be the proximal cause for dispersal (Gerlach 1990, 1996).

Due to the multiple external and internal factors affecting it, there is individual variation in the timing of dispersal (Bowler and Benton 2005; Krackow 2003). It has a strong connection with changes in the weight and hormonal status of the disperser. Since emigration is risky, individuals seem to must reach a threshold level of weight before migration, to lower its risk (Belthoff and Dufty 1998; Nunes et al. 1998; Nunes and Holekamp 1996). In naked mole rats (*Heterocephalus glaber*) (O'Riain et al. 1996), and in Belding's ground squirrels (*Spermophilus beldingi*), dispersing individuals were heavier, then non dispersers (Holekamp 1984).

Social and behavioral differences, along with different mating systems can also occur between two closely related species. Adaptation to different habitats plays a more important role than phylogeny in the evolution of mating systems (Carranza 2000; Emlen and Oring 1977). Well known is the difference between the monogamous prairie vole (*Microtus ochrogaster*) and the polygamous meadow vole (*Microtus pennsylvanicus*) (Getz et al. 1981; Oliveras and Novak 1986). Among other behavioral differences, but probably as an outcome of their mating system, meadow vole females stay in the natal area, while both sexes disperse in prairie voles (McGuire et al. 1993). The genus *Mus* is a good model in comparative behavior studies because of their phylogeny is well-known (Bonhomme et al. 1984; Chevret et al. 2005; Lundrigan et al. 2002) and as the house mouse, one of the most investigated species, can be used as a reference (Ganem et al. 2005). The house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*) are both common in Hungary. Although they occur sympatric, and morphologically similar (Demeter et al. 1995), they favor different habitats, which seems to lead to different social organization, territoriality and cooperative behavior.

The house mouse is a polygamous species with social group territoriality, adapted to the human environment (Bronson 1979; Brown 1953; Crowcroft and Rowe 1963; Lidicker 1976). They breed opportunistic rather than seasonal (Bronson and Perrigo 1987). They mature and reproduce early, while showing male-biased dispersal (Gerlach 1990, 1996). Males compete for females and develop dominance-submission relationship inside the social group (Bronson 1979; Crowcroft and Rowe 1963; Lidicker 1976; Reimer and Petras 1967). Cooperation is limited to communal nursing of pups by females while sharing a common nest (Dobson and Baudoin 2002; Dobson et al. 2000; König 1993; Manning et al. 1995; Sayler and Salmon 1969).

The mound-building mice are monogamous, and they inhabit abandoned agricultural fields. Groups of 7-10 juveniles spend the unfavorable winter period in nest chambers under vast mounds (Garza et al. 1997; Naumov 1940; Orsini et al. 1983), which they construct cooperatively from soil and plant material (Simeonovska-Nikolova and Gerasimov 2000; Szenczi et al. 2011). These groups of individuals do not reproduce till the next spring, while adult mice are seldom found in these mounds. The mound-building mice is also known for

its elevated level of aggression of both gender adults (Patris et al. 2002; Simeonovska-Nikolova 2003; Suchomelova et al. 1998).

Our aim in this study was to reveal the natal dispersal process in mound-building mouse in contrast with house mouse. We focused on the timing of dispersal, the rate of dispersers, and its relation to individual conditional differences in the two species. We expected delayed dispersal in mound-building mouse, according to the communal overwintering and the delayed sexual maturity. Based on the different mating systems of the two species we assumed that in mound-building mice there would be less or no sexual dimorphism in dispersal.

We addressed the following questions:

- Do mound-building mice and house mice differ in the timing of dispersal?
- Are there sexual differences in the dispersal process of these two species?
- Are measurable individual traits connected to individual differences in timing of dispersal?

MATERIALS AND METHODS

The experimental animals were 2 or 3rd generation descendants of randomly bred wild caught animals originating from three different populations in Hungary. The mound-building mice were collected from mounds, and the house mice were trapped from nearby farms. We tested 10 litters of each species. 64 mound-building mice (30 male 34 female), and 68 house mice (36 male, 32 female) were used in total for the experiment. The mice were kept at the Biological Station of Eötvös Loránd University in Göd, Hungary, under laboratory conditions in standard polycarbonate cages (35 x 20 x 15 cm), between 18-21°C with 12:12 h reverse L/D cycle, with red light between 08:00 and 20:00 hour. We used sawdust as bedding material (LIGNOCELL from J. Rettenmaier & Söhne GmbH, Rosenberg, Germany), hay of alfalfa were provided for nest material. The animals were offered food pellets (Ssniff S8106-SO11 Spezialdiäten GmbH, Soest, Germany) and water ad libitum.

EXPERIMENTAL DESIGN

The experimental setup was based on Gerlach's (1996) dispersal apparatus used for testing house mice. The test apparatus consisted of one central polycarbonate box (60 x 40 x 35 cm) connected to two smaller boxes (30 x 21 x 10 cm) and a water basin (78 x 16 x 14 cm) by plastic tubes (diameter 3.5 cm). On the other side of the basin was the "emigration box" (30 x 21 x 10 cm). Mice could only reach the emigration box by swimming across the water basin, where the water was 8 cm deep. There was a cylinder in every central box filled with hay for nesting (diameter 14 cm, height 7 cm). All boxes were covered with wire mesh for observation and sufficient airflow. Bedding and nesting material, ad libitum water and food were provided in all boxes including the emigration box. (Figure II/3-1) In our previous pilot study we found no difference in the swimming disposition between the two species while housed alone.

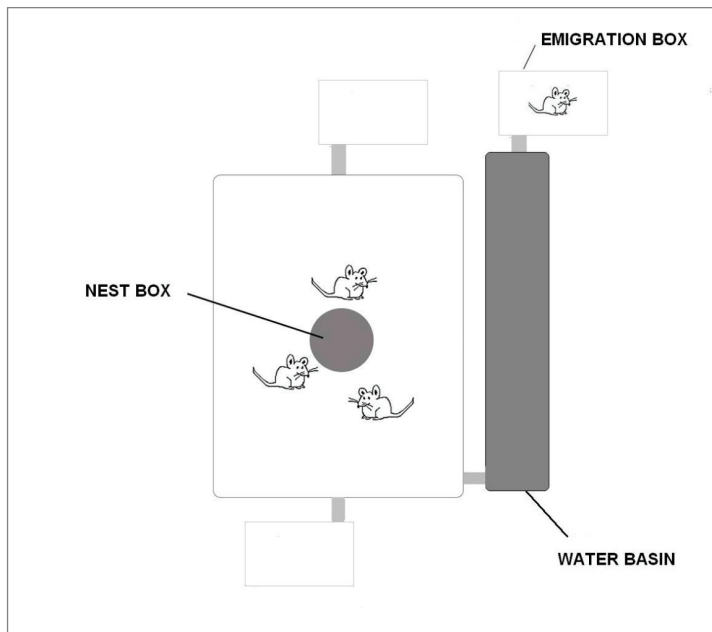


FIGURE II/3-1 SCHEMATIC PICTURE OF THE EXPERIMENTAL APPARATUS.

Whole litters without parents were placed in the central box of the apparatus at weaning (21 days). The number of animals, and the sex ratios were not modified. All animals were marked individually by fur cutting thus making it possible to identify animals by visual observation. Weaning weights were registered a priori the experiment. After placing the test animals in the central box, we kept all tubes closed for 3 days of comforting time. After opening of the gates, we checked the emigration boxes every morning. If a mouse was found in the emigration box, we identified the individual, and placed it back to the central box. If an individual was found in the emigration box on two consecutive days, it was considered an emigrant. Their weight, and age was then recorded, and the animal was removed from the apparatus. The emigration box was cleaned and replaced. The test was running until all individuals emigrated or the animals reached the age of 120 days. Animals did not disperse before this time, were considered as residents. We used 9 identical apparatus, placed in the same test room where the conditions were as during housing. Both species were tested simultaneously, with random distribution of species among the 9 apparatus

DATA ANALYSIS

Variables were tested for normal distribution with the Kolmogorov-Smirnov test and Levene's test was used to analyze homogeneity of variances. We compared the age of offspring dispersal using mixed effects Cox survival model implemented in the R package 'coxme' (Therneau 2011). Species and sex were categorical predictor with litter as random factor. Dispersal of mice was considered as the terminal event. Individuals who did not migrated till the age of 120 days were marked as censored subjects. To compare the ratio of dispersers we used mixed effect general linear model with species and sex as categorical predictor and litter as random factor. Duncan's post-hoc test was used to mark significant differences. Weaning weights of first and last dispersers from each litter were compared by paired t-tests. In case of more than one animal remained as resident, their averaged weaning weight was used.

RESULTS

AGE AT DISPERSAL

House mice and mound-building mice differed in the age of dispersal, as house mice generally left the device earlier. The mean age of dispersal in house mice males was $49.4 \text{ days} \pm 6.2 \text{ (SE)}$, and $71 \pm 7.5 \text{ (SE)}$ in females. Whereas it was $98 \text{ days} \pm 4.0 \text{ (SE)}$ in mound-building mice males and $103.5 \text{ days} \pm 3.6 \text{ (SE)}$ in mound-building mice females. Cox's mixed effect survival model revealed significant differences among the groups ($\chi^2(4) = 41.99, P < 0.0001$; Species: $\text{coef} = -2.16, \text{se} = 0.47, z = -4.56, P < 0.001$; Sex: $\text{coef} = -1.27, \text{se} = 0.40, z = -3.18, P < 0.01$, Species*Sex: $\text{coef} = 1.05, \text{se} = 0.53, z = 1.99, P < 0.05$). (Figure II/3-2)

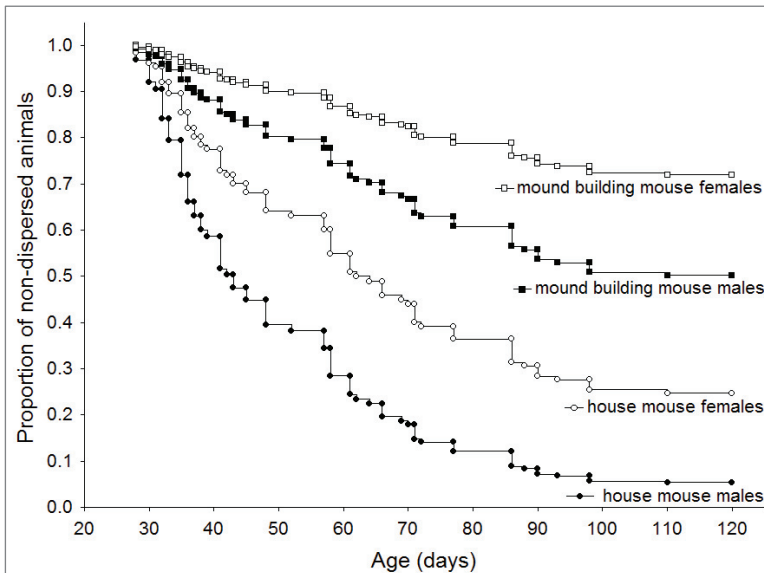


FIGURE II/3-2 RATIO OF NON-DISPersed INDIVIDUALS DURING THE TEST. SOLID CIRCLES REPRESENT HOUSE MOUSE MALES, EMPTY CIRCLES REPRESENT HOUSE MOUSE FEMALES, SOLID SQUARES REPRESENT MOUND-BUILDING MOUSE MALES, EMPTY SQUARES REPRESENT MOUND-BUILDING MOUSE FEMALES.

RATIO OF DISPERSERS

During the 100 days of the test, house mice left the apparatus in a higher ratio than mound-building mice. (Mixed effect GLM; species: $F(1,36) = 43.74$; $P < 0.0001$). There was neither significant difference between the sexes (Mixed effect GLM; sex: $F(1,36) = 1.47$; $P = 0.23$) nor in the interspecies pattern (Mixed effect GLM; species*sex: $F(1,36) = 2.09$; $P = 0.16$). (Figure II/3-3)

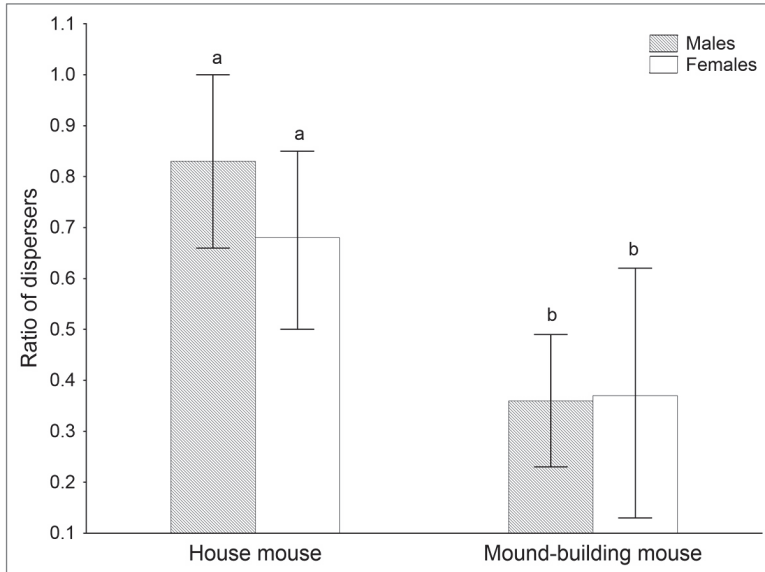


FIGURE II/3-3 RATIO OF DISPERSED ANIMALS DURING THE TEST. SOLID BARS REPRESENT MALES AND OPEN BARS REPRESENT FEMALES. THE DIFFERENT LETTERS ABOVE THE COLUMNS MARK SIGNIFICANT DIFFERENCES BY A POST HOC DUNCAN TEST.

THE ROLE OF CONDITION IN THE TIMING OF DISPERSAL

To evaluate the effect of condition on the age of dispersal, we compared the first and the last disperser's weaning weight in each litter. Our results showed that the weaning weight of individuals of this two species had different effect on the dispersal. The first disperser house mouse males were lighter at weaning than the last dispersers, or those who stayed in

the apparatus as residents (paired t -test: $t_9 = 2.69$; $P = 0.02$). There was no similar difference in the case of mound-building mouse males (paired t -test: $t_9 = 0.55$; $P = 0.59$). ([Figure II/3-4](#))



FIGURE II/3-4 WEANING WEIGHTS OF THE FIRST AND LAST DISPERSER MALES. SOLID BARS REPRESENT FIRST DISPERSERS AND OPEN BARS REPRESENT LAST DISPERSERS OR RESIDENTS. ASTERISK MARK SIGNIFICANT DIFFERENCE ($P < 0.05$) REVEALED BY PAIRED T-TEST.

Weaning condition also influenced the dispersal age of house mouse females. In case of house mouse females, the heavier individuals at weaning stayed longer in the experimental apparatus (paired t -test $t_9 = 3.61$; $P < 0.01$). Likewise as the males, we found no such difference in mound-building mouse females (paired t -test $t_9 = 0.36$; $P = 0.72$). ([Figure II/3-5](#))



FIGURE II/3-5 WEANING WEIGHTS OF THE FIRST AND LAST DISPERSER FEMALES. SOLID BARS REPRESENT FIRST DISPERSERS AND OPEN BARS REPRESENT LAST DISPERSERS OR RESIDENTS. ASTERISK MARK SIGNIFICANT DIFFERENCE ($P < 0.05$) REVEALED BY PAIRED T-TEST.

DISCUSSION

We found differences in the timing of dispersal between the two species: house mice emigrated earlier than the mound-building mice. The rate of dispersers during the test was also higher in house mice. House mice showed sexual asymmetry in dispersal: house mice males left the apparatus earlier than house mice females. Mound-building mice showed no sex bias in dispersal behavior. Weight at weaning was related to the timing of dispersal in house mice but not in mound-building mice.

Natal dispersal in mound-building mice seems to happen later than in house mice, presumably because of the communal overwintering and delayed maturation. As a result of the high mortality rate and the fast reproduction of the house mouse, an individual's best strategy to mature, disperse and find a suitable habitat for reproduction as soon as possible.

However it is also influenced by social cues. According to Gerlach (1996) dominant males expel their mature sons, which is the main cause of their natal dispersal. If not the father, one of the sons takes his place. Young males can choose between two strategies: they mature early, grow fast and fight with the father for the dominant position, or delay their maturation and wait until they reach a better physiological condition to be able to be dominant. Mound-building mice spend the winter in relatively safe environment, benefiting from the thermal advantages of group living and the insulating capacities of the mound. Field studies confirm that the mortality rate of mound-building mice is relatively low during winter (Szenczi et al. 2011). Hence, the mound-building mice born in late summer delay their sexual maturity (Gouat et al. 2003a), participate in the cooperative effort of mound-building and only disperse next spring.

As their polygamous mating system suggest house mice showed male biased dispersal, which occurs before reproduction at 2-3 month of age (Gerlach 1996; Lidicker 1976; van Zegeren 1980). These results coincide with our hypothesis and formal studies. The lack of sexual dimorphism in the dispersal of mound-building mice is also in agreement with their overwintering strategy and strengthens the hypothesis of monogamy, since monogamous mammals generally show less sexual dimorphism (Kleiman 1977). Under laboratory condition, mound-building mice show mating preference and strong pair bond typical for monogamy (Baudoin et al. 2005; Patris and Baudoin 1998). The aggression between adult females (Patris et al. 2002; Simeonovska-Nikolova 2003) also suggest monogamous mating system. However, other studies doubt the exclusive monogamy (Gomendio et al. 2006; Gouat et al. 2003b).

Individual variance in timing of dispersal can have several components, including heritable social behavior (Krackow 2003), actual condition and hormonal status (Dufty and Belthoff 2001). Since emigration is risky, individuals seem to must reach a threshold level of weight before migration, to decrease its costs (Belthoff and Dufty 1998; Nunes et al. 1998; Nunes and Holekamp 1996). In our study, we focused on the role of weight at weaning in the timing of dispersal. Dominant males are heavier than subordinate ones (Wolff 1985) and they mate with several females within their territories. We found differences only in house mice, where individuals with smaller weights left earlier. Although the same differences were found in both sexes of house mice, the causes can be different, according to their

social system. In our case, the parents were not in the apparatus, so it seems that one of the sons took the dominant position. Smaller males may disperse, because they got expelled by the dominant ones, or they mature earlier and therefore have earlier dispersal motivation.

Mature females can delay the maturation of immature daughters or sisters by urinary cues (Drickamer 1975, 1982). Thus, for younger females dispersal is the only way to reproduce (Gerlach 1996). Because in our experiment there were only one generation present, the high rate (70%) of female dispersal can be due to other environmental factors: like space use, or resource division. Weight at weaning could not predict the individual variance in timing of dispersal in mound-building mice. For them a good condition before dispersal is more important because they live in more extreme environmental conditions.

Our study showed that dispersal occurs without parents as well, with approximately one-month difference between the two sexes. According to our results at mound-building mice dispersal occurs later, which can be due to the overwintering behavior and the delayed maturation. We can assume that dispersal in the nature occurs later than four months, since almost 70% of the mound-building mice were residents in our study. We don't know whether there is a sex-bias when the real dispersal happens in the nature, at the time when they leave the mound, around six months of age.

There is little known about the life history of the mound-building mice. During overwintering behavior, sexual maturation is suppressed by the grouping effect (Feron and Gheusi 2003), and reproduction is delayed (Naumov 1940; Orsini et al. 1983). The individuals, who spend the winter under the mounds, are immature young animals. They assumed to be born approximately end of August, and they have to delay their dispersal until next spring. There is a little chance that this generation produces the next overwintering generation. During summer time, natal dispersal should be different.

CONCLUSION

Natal dispersal in mound-building mice seems to occur later then in house mice, presumably because of the communal overwintering and delayed maturation. As the behavior of the mound-building mice is influenced by the cooperative mound building and overwintering, it can lead to better understanding of the ecological constrains of group

living and cooperation. There are no sex bias in dispersal, in contrast of the house mice which as suggested by the monogamous mating system. In the future field studies would be very important to reveal the life history of the mound-building mice, as well as for fully understanding their natal dispersal.

ACKNOWLEDGMENTS

This work was supported by the GEBACO EU FP6 Grant no. 28696 to V. Altbäcker. We thank the collaborating colleagues of the Ethology Department, especially Károly Csizmadia and András Kosztolányi, for their contribution to gathering and analyzing the data.

ETHICAL STANDARDS

The procedure used was approved by the Ethical Committee for Animal Experiments at Eötvös Loránd University, and followed the rules detailed in the guidelines of the American Society of Mammalogists (Gannon and Sikes 2007) and the European Communities Council Directive of 24 November 1986 (86/609/EEC). Animals used in this test did not participate in any other research but were used for breeding afterwards.

II/4. DEVELOPMENT OF THE SOCIAL BEHAVIOR OF TWO MICE SPECIES WITH CONTRASTING
SOCIAL SYSTEMS

Péter Szenczi*; Oxana Bánszegi; Zita Groó; Vilmos Altbäcker,

Published in: Aggressive Behavior

Volume 38, Issue 4, July/August 2012, Pages 288–297

ABSTRACT

The house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*) differ in their social system and show different types of cooperative behavior. The *M. musculus* is polygynous where females tend to breed cooperatively while *M. spicilegus* is known to be monogamous and famous for its unique cooperative behavior; the communal overwintering. *M. spicilegus* is considered as a highly aggressive species in the genus *Mus*. In the present study, we attempted to analyze the development of social behavior and the importance of familiarity between the *M. musculus* and *M. spicilegus*. To study how social system and cooperation reflects on social behavior, dyadic social interaction tests in neutral cages were performed on 21, 60 and 120 days old mice. Each individual was tested against a sibling and an unfamiliar individual at all three ages. Our results showed that social behavior and its development differed between the species and sexes. When facing a stranger, both sexes of *M. spicilegus* were more aggressive than *M. musculus*. The familiarity of the opponent was more important to *M. spicilegus* than to *M. musculus*. Despite their elevated level of aggression *M. spicilegus* remained less agonistic against their sibling, indicating that either kinship or early social experience elicit tolerance. Our study suggests that the social system of mice is reflected in their behavioral patterns, and the two contrasting species studied may provide a useful model of behavioral development.

Keywords: aggression, *Mus musculus*, *Mus spicilegus*, social behavior, social system

INTRODUCTION

Aggression plays a major role in the determination of mating systems and social organization (Trivers 1972). Comparative studies on rodents show that the level of agonistic behavior and tolerance between individuals is mostly under the influence of ecological traits and in direct connection with sociality, the presence or absence of social groups, and the breeding system (Armitage 1981; Crowcroft and Rowe 1963; Getz and Carter 1980; Getz et al. 1981; Insel et al. 1995; Livoreil et al. 1993; Patris et al. 2002; Sachser et al. 1999; Shapiro et al. 1986). Mutual tolerance between individuals is key to carry out cooperative behavior traits, while selective aggression usually directed toward strangers may be very important in maintaining territories and the social bonds, established between individuals.

Intrasexual aggression in polygynous species is usually more explicit among males and associated with competition for mating opportunities (Trivers 1972). Monogamy is usually characterized by selective affiliation with a partner, high levels of paternal behavior, and intense aggression towards strangers for defense of territory, nest, and mate (Clutton-Brock 1989; Kleiman 1977). It is important to note that in case of species that are highly adaptable, the manifest and level of their aggressive behavior might show great variability between populations and strains (Brain and Parmigiani 1990; Frynta et al. 2005; Ganem and Searle 1996; Sluyter et al. 1996; Vom Saal et al. 1995). Adaptation to different ecological conditions can result in markedly different physiology and social behavior (Bronson 1985). Individual differences such as environmental factors, hormonal stages and previous experience may also alter the behavior of animals in social situations.

The house mouse (*Mus musculus* Linnaeus 1758) is characterized by a polygynous mating system with social group territoriality (Bronson 1979; Brown 1953; Crowcroft and Rowe 1963; Lidicker 1976). They mature and reproduce early, showing male-biased dispersal (Gerlach 1996), while breeding opportunistically rather than seasonal (Bronson and Perrigo 1987). Males compete for females and develop dominance-submission relationship inside the social group (Bronson 1979; Crowcroft and Rowe 1963; Lidicker 1976; Reimer and Petras 1967). Cooperation is limited to females of certain populations and strains taking care of pups communally by sharing a common nest, and nurse each-others pups (Dobson and Baudoin 2002; Dobson et al. 2000; König 1993; Manning et al. 1995; Sayler and Salmon

1969). Hence, males are more agonistic than females, while females tolerate each other in certain circumstances.

The mound-building mouse (*Mus spicilegus* Petényi 1882) is believed to be the only species in the *Mus* complex to exhibit a mating system of social monogamy (Baudoin et al. 2005; Patris et al. 2002). They establish strong social bond between partners (Patris and Baudoin 1998), both female and male adult mice show high aggressiveness towards unfamiliar individuals (Patris et al. 2002; Simeonovska-Nikolova 2003; Suchomelova et al. 1998). They form stable male-female associations in experimental groups (Baudoin et al. 2005; Simeonovska-Nikolova 2003) while polygynous females have low reproductive success (Gouat and Feron 2005). Males and females cooperate in the parental care of their offspring (Patris and Baudoin 2000).

The unique communal overwintering phenomenon is another, or even the most important factor that influences the social behavior of the *M. spicilegus*. In contrast with the *M. musculus*, the *M. spicilegus* is not commensal, and avoid human settlements. They construct large mounds in autumn, in which seeds and other kinds of plant materials are cached, and they overwinter under these structures (Murariu 1981; Naumov 1940; Sokolov et al. 1998). Individuals inhabiting the mound are almost exclusively juveniles (Canady et al. 2009; Garza et al. 1997; Milishnikov et al. 1998; Poteaux et al. 2008; Simeonovska-Nikolova 2007). They might belong to a single litter but more commonly derived from differently related female parents and their unrelated mates (Garza et al. 1997). Overwintering individuals delay their sexual maturation, they only leave the mound at spring, when they disperse and reproduce (Milishnikov et al. 1998; Naumov 1940; Orsini et al. 1983; Simeonovska-Nikolova and Gerasimov 2000). The delay is the result of the social effects of living in groups and not the environmental conditions of winter (Feron and Gheusi 2003; Gouat et al. 2003a). The building of a mound has a high cost but it may play an important role in the success of overwintering (Szenczi et al. 2011). This kind of cooperative behavior demands that juveniles must recognize and be tolerant toward each other, but defend their mound, protect their resources from intruders.

In previous studies, the *M. musculus* male-male interactions were found to be more agonistic than the female-female ones. Females were generally tolerant to each other in

neutral cage test and they became aggressive only under certain circumstances (Frynta et al. 2005; Munclinger and Frynta 2000; Palanza et al. 1996; Parmigiani and Palanza 1994; Parmigiani et al. 1989; Patris et al. 2002). Adult *M. musculus* males mature early and are aggressive toward related and unrelated individuals as well (Rowe and Redfern 1969). Behavior tests of adult *M. spicilegus* revealed that during the reproductive period the social interactions between both sexes in encounters are characterized by high level of aggressiveness and less tolerance (Patris et al. 2002; Suchomelova et al. 1998). Nevertheless, no studies examined the behavior of juvenile *M. spicilegus* in the age when they communally use the burrow system under the mounds, which protect them from the harsh winter conditions.

In the present study, we aimed to show the differences through the development of behavior between *M. musculus* and *M. spicilegus* in intrasexual social interaction tests. We expected differences due to the a) different mating system of the two species resulting in elevated level of aggression in both sexes of the monogamous *M. spicilegus*, and b) different cooperative behavior and group forming factors, which may lead to more tolerance among familiar *M. spicilegus*. Considering that these species live under different ecological and social conditions where the behavior in various age might be different we decided to perform the tests in three ages from weaning.

MATERIALS AND METHODS

Experimental animals (both *M. musculus* and *M. spicilegus*) were randomly bred 2nd to 3rd generation descendants of wild-caught animals, originating from three different populations from Hungary. All litters used in this test were born between 13 March and 08 July 2008. Animals were housed in polycarbonate standard mouse cages (35x20x15) with wood shavings (Lignocel) as bedding material. Hay of alfalfa was provided for nesting material. Temperature was kept constant (between 18oC and 21oC) and reverse 12 L: 12 D light cycle with red light between 0800 and 2000 hours was set up. Pelleted mouse food (Ssniff s8106-s011) and water was provided ad libitum. Animals were weaned at the age of 21 days. They were housed with their littermates until the age of 40 days and kept individually afterwards. No test animals had previous sexual experience.

To test the different reactions of the individuals to a known and a stranger opponent, each individual was tested against a same sex littermate and a same sex unfamiliar individual at ages 21, 60 and 120 days. Sequence of the two opponents was randomly arranged. Thirty-seven animals were used in total (9 *M. musculus* male, 9 *M. spicilegus* male, 9 *M. musculus* female, and 10 *M. spicilegus* female). Opponents were randomly chosen before the first test from non-tested same age individuals with approximately the same weight (+/- 10 %), who were treated and housed similarly to test animals. Test animals and opponents were marked with hair clipping before each session.

Behavioral sciences developed several methods for studying social behavior, since the differences between species are context-dependent in many cases. We used a standard neutral-cage procedure for testing social interactions following the method used by Patris et al (2002). While results may differ between test situations, the neutral cage social interaction test, where dyadic encounters are monitored, is a sensitive and widely used tool for studying aggression and tolerance. The few studies on the social behavior of *M. spicilegus* used similar methods, thus, the results can be compared to previous experiments. The tests were carried out in a 50 x 30 x 35 cm glass cage. The cage was divided into two equal parts by a plastic card partition. During the tests, a single 40 W red light bulb illuminated the cage. The mice were tested in the dark phase of their light-dark cycle between 0900 and 1200. At the beginning of each test, the opponents were placed in the opposite sides of the cage, and left undisturbed for five minutes. The central partition was then removed and video recording by a single VHS-camera started. The tests started when one or both animals approached the other for the first time and lasted 10 minutes. After each test, the cage was thoroughly cleaned using 70% ethanol and left to dry out completely. Four identical cages were used in rotation. Tests of the same individual occurred at least 24 hours apart.

We measured the time that the animals had spent with agonistic (either offensive or defensive) and sociable behavioral elements following Patris et al. (2002). The behavioral repertoire was established based on previous studies on mice (Frynta et al. 2005; Grant and Mackintosh 1963; Patris et al. 2002). Observed behavior units were thus grouped into sociable behaviors (attend, approach, nose, follow, sniff, investigate, grooming) and agonistic behaviors (offensive upright posture, threat, boxing, fighting, thrust, chasing,

defensive upright posture, retreat, evade, flee, and crouching posture). Latencies of first approach and first agonistic interactions were also recorded. Where no agonistic interaction was recorded, we used 600 sec. (length of test) as latency of the first attack. The effects of sex, age and familiarity on time spent by agonistic and sociable behaviors were evaluated.

DATA ANALYSIS

Data were analyzed using Statistica 8.0 statistical software (StatSoft 2007). Male-male and female-female dyads were examined separately by two ways (species and familiarity as factors) repeated measures (age as within subject factor) General Linear Models (GLM). All data were log transformed to meet conditions of normality for statistical purposes. One second was added to all data before the transformation in order to substitute zeros. To mark significant differences Duncan's Post-hoc tests were used, making all pairwise comparisons separately in each age group, following repeated measures GLMs with familiar-unfamiliar repeats (R) as within subject factor. Spearman rank-order correlation (r_s) was used to evaluate the relationship between the latency of the first agonistic interaction and the time spent in agonistic behavior.

ANIMAL TREATMENT

The procedure used was approved by the Ethical Committee for Animal Experiments at Eötvös Loránd University, and followed the rules detailed in the guidelines of the American Society of Mammalogists (Gannon and Sikes 2007) and the European Communities Council Directive of 24 November 1986 (86/609/EEC). The tests were continuously monitored via a TV screen from another room in order to intervene in case of injuries. Tests were to suspend if in case one of the animals was unable to flee or evade its opponent, wounded from biting or suffered other injuries. From the 222 encounters on two occasions (1 *M. spicilegus* male 120 days test with unfamiliar opponent, 1 *M. musculus* male 120 days with familiar opponent) we had to stop the tests because one of the animals got bitten in the paw and started bleeding. Injuries were treated with Betadine antiseptic solution (EGIS PLC. Budapest, Hungary) and both individuals were placed back to their cages. No fatal or serious injuries occurred during the test. Animals used in this test did not participate in any other research but were used for breeding afterwards.

RESULTS

MALES' SOCIABLE BEHAVIOR

We found differences in the sociable behavior of males. The amount of time spent in sociable behavioral elements decreased with age in both species. Decrease of time spent in sociable behavior was more explicit in *M. musculus* than *M. spicilegus*. Time spent in sociable behavioral elements dropped to almost zero in case of the *M. musculus* at the test 120 days of age, while they differentiated between siblings and strangers only at the age of 60 days, by being more amicable with siblings. The time that *M. spicilegus* males spent on sociable behavioral elements was more constant during development. At the age of 120 days they spent significantly more time with amicable behavior than *M. musculus* males ([Figure II/4-1](#)). See [Table II/4-1](#) for details of statistical analysis.

MALES' AGONISTIC BEHAVIOR

There were differences in the agonistic behavior of males. Time spent in agonistic behavior increased with age in both species. The familiarity of animals had an effect and it differed among the compared species. The pattern of development of agonistic behavior did differ and age had different effect on discrimination. *M. musculus* males started to show signs of agonistic behavior at the age of 60 days, by spending more time fighting with strangers. At the age of 120 days their level of aggression was elevated without distinguishing siblings. Agonistic behavior of *M. spicilegus* males appeared earlier but only against strangers. At the age of 120 days, they were highly agonistic toward strangers, but remained tolerant to their siblings. ([Figure II/4-2](#)). See [Table II/4-1](#) for details of statistical analysis.

TABLE II/4-1

RESULTS OF STATISTICAL ANALYSIS ON THE TIME SPENT IN SOCIABLE AND AGONISTIC BEHAVIORAL ELEMENTS. MALE-MALE AND FEMALE-FEMALE DYADS WERE EXAMINED SEPARATELY BY TWO WAYS (SPECIES AND FAMILIARITY AS FACTORS) REPEATED MEASURES (AGE AS WITHIN SUBJECT FACTOR) GENERAL LINEAR MODELS.

Male's sociable behavior	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>p</i>
AGE	16.08	2	8.04	120.11	<0.001
AGE*Species	10.03	2	5.02	74.94	<0.001
AGE*Familiarity	0.23	2	0.11	1.70	0.191
AGE*Species*Familiarity	0.70	2	0.35	5.22	0.008
Error	4.28	64	0.07		
Female's sociable behavior	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>p</i>
AGE	1.80	2	0.90	38.54	<0.001
AGE*Species	0.10	2	0.05	2.09	0.131
AGE*Familiarity	0.23	2	0.12	5.01	0.009
AGE*Species*Familiarity	0.46	2	0.23	9.77	<0.001
Error	1.59	68	0.02		
Male's agonistic behavior	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>p</i>
AGE	46.46	2	23.23	135.59	<0.001
AGE*Species	3.73	2	1.86	10.88	<0.001
AGE*Familiarity	2.83	2	1.42	8.27	0.001
AGE*Species*Familiarity	1.62	2	0.81	4.73	0.012
Error	10.96	64	0.17		
Female's agonistic behavior	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>p</i>
AGE	10.04	2	5.02	25.91	<0.001
AGE*Species	0.43	2	0.22	1.11	0.334
AGE*Familiarity	10.04	2	5.02	25.91	<0.001
AGE*Species*Familiarity	0.43	2	0.22	1.11	0.334
Error	13.18	68	0.19		

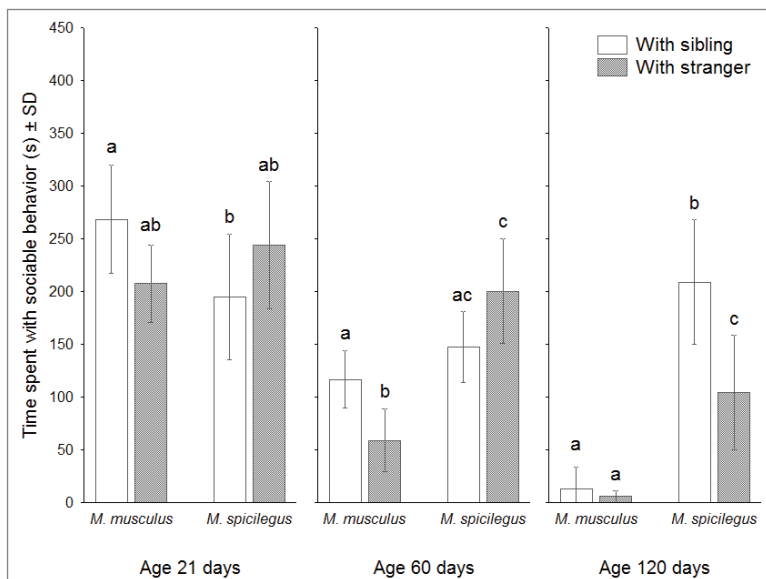


FIGURE II/4-1 TIME SPENT IN SOCIABLE BEHAVIOR (MEAN \pm SD) BY MALES. OPEN BARS REPRESENT ENCOUNTERS WITH FAMILIAR INDIVIDUALS AND SOLID BARS REPRESENT ENCOUNTERS WITH UNFAMILIAR INDIVIDUALS. THE DIFFERENT LETTERS ABOVE THE COLUMNS MARK SIGNIFICANT DIFFERENCES BY A POST HOC DUNCAN TEST PERFORMED SEPARATELY FOR THAT PARTICULAR AGE CATEGORY (REPEATED MEASURES GLM, AGE 21: SPECIES: $F(1, 16) = 0.75$, $P = 0.39$; $R F(1, 16) = 0.01$, $P = 0.90$; SPECIES* $R F(1, 16) = 7.35$, $P = 0.015$; AGE 60: SPECIES $F(1, 16) = 44.0$, $P < 0.001$; $R F(1, 16) = 3.78$, $P = 0.069$; SPECIES* $R F(1, 16) = 16.49$, $P < 0.001$; AGE 120 SPECIES $F(1, 16) = 72.17$, $P < 0.001$; $R F(1, 16) = 4.87$, $P = 0.042$; SPECIES* $R F(1, 16) = 2.47$, $P = 0.13$)

FEMALES' SOCIABLE BEHAVIOR

We found species-specific differences in the social behavior of females. In most cases, *M. spicilegus* females spent the same amount or more time with sociable behavior as *M. musculus*. Social behavior changed during development in both species. The familiarity of opponents had effect. The mice spent more time socializing with their siblings. *M. musculus* females spent less time with strangers from the age of 60 days while *M. spicilegus* spent significantly more time with familiars when 120 days old. (Figure II/4-3). See Table II/4-1 for details of statistical analysis.

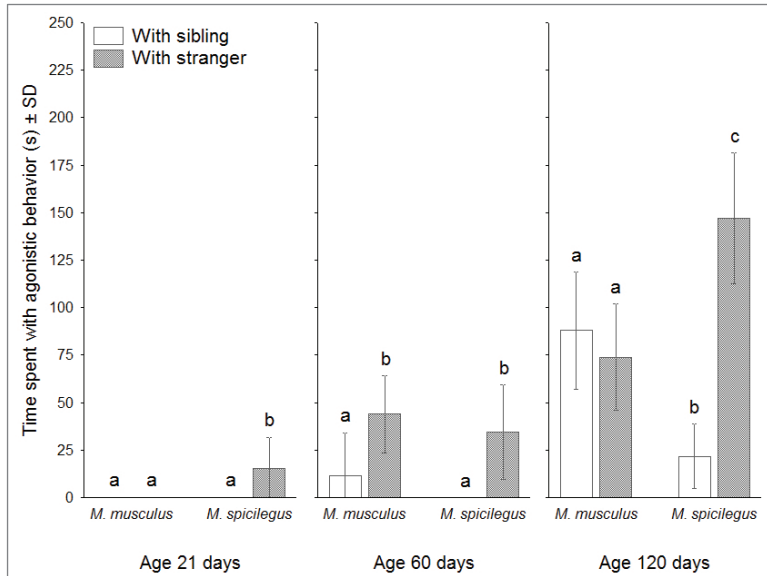


FIGURE II/4-2 TIME SPENT IN OFFENSIVE AND DEFENSIVE BEHAVIOR (MEAN \pm SD) BY MALES. OPEN BARS REPRESENT ENCOUNTERS WITH FAMILIAR INDIVIDUALS AND SOLID BARS REPRESENT ENCOUNTERS WITH UNFAMILIAR INDIVIDUALS. THE DIFFERENT LETTERS ABOVE THE COLUMNS MARK SIGNIFICANT DIFFERENCES BY A POST HOC DUNCAN TEST PERFORMED SEPARATELY FOR THAT PARTICULAR AGE CATEGORY (REPEATED MEASURES GLM, AGE 21: SPECIES: $F(1, 16) = 9.76$, $P = 0.016$; $R F(1, 16) = 9.81$, $P < 0.01$; SPECIES* $R F(1, 16) = 9.81$, $P < 0.01$; AGE 60: SPECIES $F(1, 16) = 6.71$, $P = 0.019$; $R F(1, 16) = 39.6$, $P < 0.001$; SPECIES* $R F(1, 16) = 0.11$, $P = 0.74$; AGE 120 SPECIES $F(1, 16) = 4.08$, $P = 0.060$; $R F(1, 16) = 15.8$, $P < 0.001$; SPECIES* $R F(1, 16) = 21.2$, $P < 0.001$).

FEMALES' AGONISTIC BEHAVIOR

Agonistic behavior of females changed during development in both species. Familiarity of animals also had effect. Neither of the two species' females showed agonistic behavior with their siblings through our tests. Time of agonistic behavior toward strangers greatly elevated at the age of 120 days, when the females of both species became highly agonistic but only with strangers. *M. spicilegus* females were even more agonistic toward unfamiliar individuals than *M. musculus* females. (Figure II/4-4). See Table II/4-1 for details of statistical analysis.

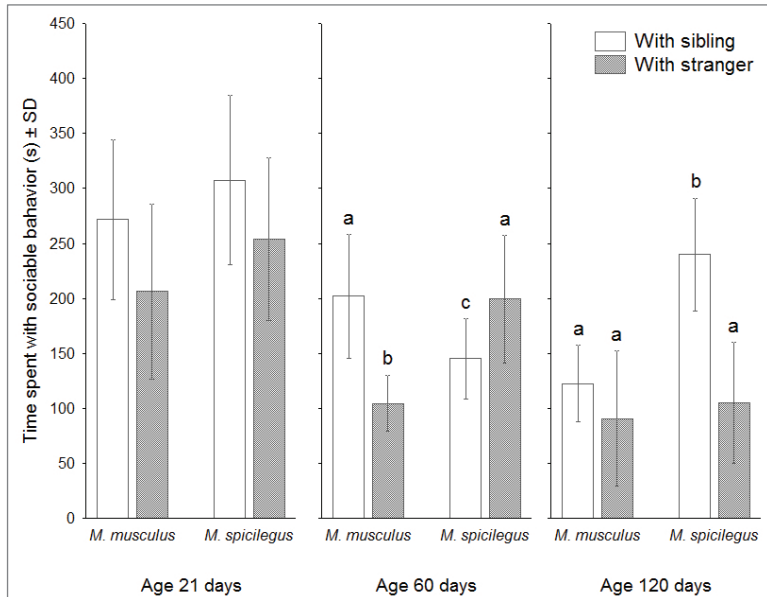


FIGURE II/4-3 TIME SPENT IN SOCIAL BEHAVIOR (MEAN \pm SD) BY FEMALES. OPEN BARS REPRESENT ENCOUNTERS WITH FAMILIAR INDIVIDUALS AND SOLID BARS REPRESENT ENCOUNTERS WITH UNFAMILIAR INDIVIDUALS. THE DIFFERENT LETTERS ABOVE THE COLUMNS MARK SIGNIFICANT DIFFERENCES BY A POST HOC DUNCAN TEST PERFORMED SEPARATELY FOR THAT PARTICULAR AGE CATEGORY (REPEATED MEASURES GLM, AGE 21: SPECIES: $F(1, 17) = 1.70$, $P = 0.21$; $R F(1, 17) = 5.38$, $P = 0.033$; SPECIES* $R F(1, 17) = 0.26$, $P = 0.62$; AGE 60: SPECIES $F(1, 17) = 2.74$, $P = 0.12$; $R F(1, 17) = 3.03$, $P = 0.10$; SPECIES* $R F(1, 17) = 22.9$, $P < 0.001$; AGE 120 SPECIES $F(1, 17) = 11.2$, $P = 0.004$; $R F(1, 17) = 26.4$, $P < 0.001$; SPECIES* $R F(1, 17) = 3.52$, $P = 0.078$).

LATENCY OF FIRST APPROACH

The time it took the mice to contact each other at the beginning of the test varied greatly, mostly within the groups. Generally, it took less time as the mice got older, but we found no clear differences between species and acquaintances. (Table II/4-2)

LATENCY OF FIRST AGONISTIC INTERACTION

The latencies of the first agonistic interactions correlates with the previous results on the time spent in agonistic interactions in all age categories (Age 21: $r_s = -0.583$, $n = 74$, $p < 0.001$; Age 60: $r_s = -0.730$, $n = 74$, $p < 0.001$; Age 120: $r_s = -0.602$, $n = 74$, $p < 0.001$). Generally, both sexes of *M. spicilegus* spent more time with introductory, sociable behavioral elements before the first attack than *M. musculus*, even in cases followed by highly agonistic interactions. Whereas, in case of *M. musculus* agonistic encounters took place almost immediately after the first encounter (Table II/4-3)

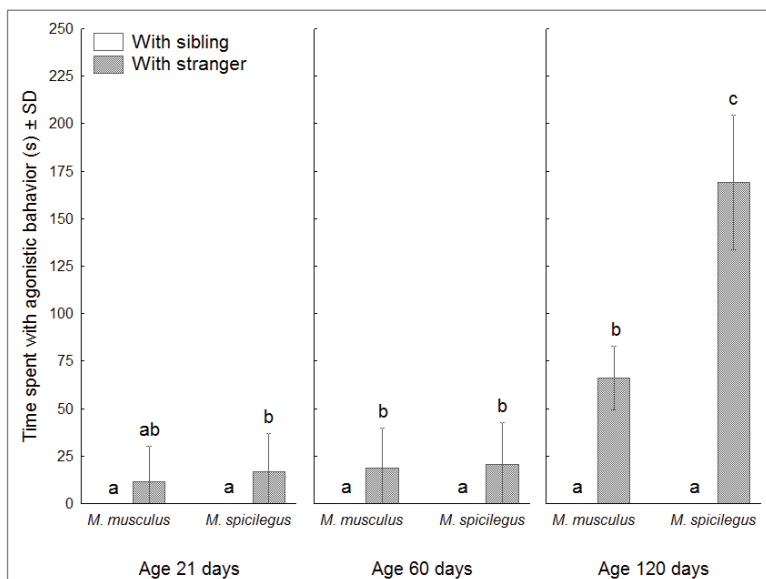


FIGURE II/4-4 TIME SPENT IN OFFENSIVE AND DEFENSIVE BEHAVIOR (MEAN \pm SD) BY FEMALES. OPEN BARS REPRESENT ENCOUNTERS WITH FAMILIAR INDIVIDUALS AND SOLID BARS REPRESENT ENCOUNTERS WITH UNFAMILIAR INDIVIDUALS. THE DIFFERENT LETTERS ABOVE THE COLUMNS MARK SIGNIFICANT DIFFERENCES BY A POST HOC DUNCAN TEST PERFORMED SEPARATELY FOR THAT PARTICULAR AGE CATEGORY (REPEATED MEASURES GLM, AGE 21: SPECIES $F(1, 17) = 0.52$, $P = 0.48$; R $F(1, 17) = 11.8$, $P = 0.003$; SPECIES*R $F(1, 17) = 0.43$, $P = 0.52$; AGE 60: SPECIES $F(1, 17) = 0.27$, $P = 0.06$; R $F(1, 17) = 32.3$, $P < 0.001$; SPECIES*R $F(1, 17) = 0.32$, $P = 0.58$; AGE 120 SPECIES $F(1, 17) = 64.7$, $P < 0.001$; R $F(1, 17) = 6719$, $P < 0.001$; SPECIES*R $F(1, 17) = 65.8$, $P < 0.001$).

TABLE II/4-2

LATENCY OF THE FIRST APPROACH (S) \pm SD. MALES: GLM AGE* SPECIES* FAMILIARITY $F(2, 64) = 5.098, P < 0.01$; FEMALES: GLM AGE* SPECIES* FAMILIARITY $F(2, 64) = 1.338, P = 0.27$

	Age 21 sibling	Age 21 stranger	Age 60 sibling	Age 60 stranger	Age 120 sibling	Age 120 stranger
M. musculus male	40.4 \pm 47.2	26.3 \pm 36.7	22.0 \pm 34.0	18.7 \pm 21.7	1.6 \pm 0.7	10.6 \pm 13.0
M. spicilegus male	27.1 \pm 47.3	91.2 \pm 62.5	11.4 \pm 8.0	6.8 \pm 3.4	3.4 \pm 3.3	16.7 \pm 21.6
M. musculus female	111 \pm 77	86.3 \pm 44.2	35.8 \pm 18.1	27.9 \pm 49.1	6.0 \pm 5.1	20.2 \pm 34.8
M. spicilegus female	25.1 \pm 30.6	66.6 \pm 65.5	36.4 \pm 48.7	19.0 \pm 27.1	10.8 \pm 18.6	6.1 \pm 10.2

TABLE II/4-3

LATENCY OF THE FIRST AGONISTIC INTERACTION (S) \pm SD. MALES: GLM AGE* SPECIES* FAMILIARITY $F(2, 64) = 9.964, P < 0.001$; FEMALES: GLM AGE* SPECIES* FAMILIARITY $F(2, 64) = 8.769, P < 0.001$

	Age 21 sibling	Age 21 stranger	Age 60 sibling	Age 60 stranger	Age 120 sibling	Age 120 stranger
Mus musculus male	600 \pm 0	600 \pm 0	456 \pm 204	84.1 \pm 53.6	13.2 \pm 21.2	2.8 \pm 1.6
Mus spicilegus male	600 \pm 0	510 \pm 177	600 \pm 0	427 \pm 259	297 \pm 175	38.1 \pm 21.2
Mus musculus female	600 \pm 0	524 \pm 116	600 \pm 0	285 \pm 66	600 \pm 0	19.9 \pm 17.1
Mus spicilegus female	600 \pm 0	451 \pm 228	600 \pm 0	498 \pm 195	600 \pm 0	110 \pm 49.8

DISCUSSION

By following intraspecific dyadic encounters of two closely related species of mice tested at different ages in neutral cage encounters, we examined how development of social behavior and discrimination reflects the differences in their social system and cooperative behavior. Our results showed that the social behavior of this two species was different. The development of social behavior differed between the species and sexes, as well as the role of familiarity.

In general, time spent in agonistic behavior increased while amount of sociable behavior decreased with age in both species. In the *M. spicilegus*, time in agonistic behavior increased more rapidly and they become more aggressive than *M. musculus* regarding the time they spent in agonistic behavioral elements. At the age of 120 days, both *M. spicilegus* males and females were more aggressive than *M. musculus* when faced an unfamiliar partner but amount of sociable behavior were more stable, probably due to longer introductory behavior.

Despite the considerable individual variation in the latency of the first agonistic interactions at the 60 and 120 days test, the introductory periods of *M. spicilegus* were generally longer. It might have been connected to the more explicit behavioral response difference with familiar vs. non-familiar opponents. Probably it takes more time for an individual to ascertain the identity of the opponent when distinguishing familiars or kins is of special importance.

Familiarity played more important role in *M. spicilegus* than in *M. musculus*. *M. musculus* distinguished siblings and strangers in young age, being more sociable or less agonistic with their siblings. Adult *M. musculus* males did not distinguish between siblings and unfamiliar, and only females tended to be more tolerant toward their siblings when reached adulthood, while still aggressive with strangers. It is known from previous studies that cooperation among *M. musculus* is limited to females and related individuals breed communally more often and more successfully than unrelated ones (König 1994; Manning et al. 1995; Manning et al. 1992; Wilkinson and Baker 1988). *M. spicilegus* were less aggressive toward siblings with whom they were raised together. At the age of 60 days, they attacked only nonfamiliar

individuals, and at the age of 120 days, they were still tolerant toward their sibling although they were separated for more than 80 days.

These differences are coherent with their different social system and ecology. Polygamy implicates high level of male competition and dispersal, and the behavioral characteristic of *M. musculus* match these criteria (Crowcroft and Rowe 1963; Gerlach 1996; Lidicker 1976; Patris and Baudoin 2000; Patris et al. 2002). However, monogamy implicates high level of competition between females as well (Dobson and Jones 1985; Favre et al. 1997; Komers and Brotherton 1997) because female aggression prevents aggregation and lead to female dispersal. In our study, we demonstrated elevated level of aggression between adult *M. spicilegus* males and females as found in previous studies (Patris et al. 2002; Simeonovska-Nikolova 2003; Suchomelova et al. 1998). Although, our experiment did not last long enough to show when siblings, especially females, become intolerant toward each other.

The observed tolerance of *M. spicilegus* with their siblings explains how they can form and live in large groups during their communal overwintering phase and how this otherwise highly agonistic species is capable of long-term cooperative behavior, which requires tolerance among individuals. The overwintering mice are approximately at the same age (Canady et al. 2009; Garza et al. 1997; Milishnikov et al. 1998; Poteaux et al. 2008; Simeonovska-Nikolova 2007), born in late summer-early autumn and during the winter (Naumov 1940; Orsini et al. 1983; Pisareva 1948). The high cost and the role of the structure in their overwintering success makes it necessary for the inhabitants to be tolerant toward each other, but defend their mound from intruders. The ability to recognize kin is widespread among rodents (Mateo 2003), but the mechanisms behind it can vary between the taxa. Animals can recognize each other indirectly from spatial cues (Holmes and Sherman 1982) or the recognition can be based on familiarity via prior association. Our study was unable to determine the basics of this behavioral trait, whether it is based on genetic similarity or familiarity.

It must be noted that in different test situation such as colony and dyad resident-intruder test or sensory contact models, the experienced level of aggression may be different. The pattern of aggressive behavior, the relative occurrences of traits can vary among different

contexts as well as hormonal and social status of the individuals (Adams 1980; Pellis et al. 1992).

A well-known example for closely related species with different mating system, and social behavior is the monogamous California mouse (*Peromyscus californicus*), a species in which both sexes are highly aggressive and provide extensive parental care toward offspring (Bester-Meredith et al. 1999; Dudley 1974; Gubernick and Alberts 1987; Trainor and Marler 2001), and the white-footed mouse (*Peromyscus leucopus*), where males and females show less aggression and parental care (Bester-Meredith et al. 1999; Korytko and Vessey 1991; Metzgar 1971; Schug et al. 1992; Wolff and Cicirello 1990). Many comparative studies have been made on these species in the last decades. The *Mus* group is also a good model for comparative behavioral studies on species that exhibit different mating systems because the phylogeny of this group is well known (Bonhomme et al. 1984; Boursot et al. 1993; Chevret et al. 2005; Sage et al. 1993) while different species of the group have been studied in various habitats, and one species, the *M. musculus*, has been studied extensively and can be used as a reference (Ganem et al. 2005).

Definite statements on a species' social behavior cannot be drawn from one type of test, but our study shows that the social system of mice is also reflected in their behavioral patterns, and the two contrasting species studied may provide a useful model of behavioral development and the behavioral bases of cooperation.

ACKNOWLEDGEMENTS

This work was supported by the GEBACO EU FP6 Grant no. 28696 to V. Altbäcker. We thank the collaborating colleagues of the Ethology Department especially Károly Cszimadia and András Kosztolányi for their contribution in gathering and analyzing the data. We thank Márta Antal for suggestions regarding the manuscript.

Chapter III.

GENERAL DISCUSSION

MOUNDS AS INSULATORS

Mounds of the mound-building mice are large constructions, especially when compared to the size of the mice. Their total volumes vary among habitats but can reach 300 liters. I found mound sizes consistent with reports of other studies (Hözl et al. 2009; Pisareva 1948; Sokolov et al. 1990). The mounds always contain significant amount of plant material which is covered by a soil layer. We usually found only one nest chamber per mound, whose depth was determined by the physical characteristics of the soil. It was always beneath the mound but we could not find any system in its position. Nonetheless I did not examine this, I presume that the mice construct the nest first or it is their natal nest and build the mound above it. The number of inhabiting mice in my study was more constant than other authors reported (Canady et al. 2009; Garza et al. 1997; Gouat et al. 2003a; Hözl et al. 2009; Muntyanu 1990; Poteaux et al. 2008). This is probably due to the new method we used to excavate the nests, which provides more reliable information on the number of animals and the composition of the groups. In agreement with other studies I barely found adult mice under the mounds. The parents may either die till the winter, or they do not build mounds for overwintering. Despite other researcher's results (Hözl et al. 2009) the mound size and plant fill volume in my study correlated positively with the number of mice inhabiting them. Consequently the quality of the mounds, as a result of a cooperative action depends on the joint work of the participating individuals.

Several authors reported that the vegetable material collected by the mound-building mice for their mound does not completely reflect its availability in the environment (Hözl et al. 2009; Hözl et al. 2011b). In most cases the hoarded plants represented only a few species. It is clear from the field data that the mice selectively use certain species for different purposes. According to my results mice mostly consume and build the mounds using dicots but the species used for the two different roles are not identical but all are parts of common weeds associated with initial successional stages on abandoned land. However, for nest material monocots were used almost exclusively.

The mounds of the mound-building mice were treated in the literature as food storage facilities but no further investigations were performed to validate this belief (Naumov 1940; Pisareva 1948; Sokolov et al. 1998). Bihari (2004a) assumed that mounds may have a role other than to serve purely as food cache and suggested other explanations like thermal insulation, water proofing or heat production. By investigating the food composition of the mice's diet, based on their fecal pellets, I found no evidence that they consume the hoarded seeds during the winter. However, this possibility cannot be completely excluded; there can be time periods or environmental conditions under which the mice eat the collected seeds. In my studies I found evidence that the mounds have thermal insulating and water proofing properties (Szenczi et al. 2011; Szenczi et al. 2012). The mounds reduce the thermal variation of the soil beneath, and elevated temperatures were measured in the soil but not in the mounds. We found no evidence that the vegetable material produces heat by rotting. Water content of the soil was also reduced under the mounds, verifying that the mounds have water proofing abilities. The plant material layer of the mounds plays an important role in thermal insulation and water proofing. Mounds with plant layer performed better in these tasks both under field conditions and in manipulated experiment.

OVERWINTERING IS SUCCESSFUL

Populations of small mammals generally decrease in the winter (Aars and Ims 2002; Berry et al. 1973; Bradshaw 1992; Gipps et al. 1985; Pucek et al. 1993). During our survey (Szenczi et al. 2011) we did not find significant decline in the number of mound-building mouse inhabitants of the mounds during the cold months. It is most likely that nest and mound building in the autumn, supported by the thermoregulatory benefits of huddling, increases the probability of overwintering of the mound-building mice. In temperate and subarctic climates even small energy savings influence survival (Geiser 1998; Körtner and Geiser 2000; Lovegrove et al. 2001; Németh et al. 2009). The advantages of group living, the access to a well-insulated underground nest, protection from moisture and thermal extremes provided by the mound, allows for energy conservation and thereby increases winter survival. This decreased mortality can outbalance the reduced reproduction rate of the mound-building mice, which is the result of the extremely delayed maturity of this species (Feron and Gheusi 2003; Gouat et al. 2003a). Since the mounds are huge structures compared to the size of one individual and last only for one season, building them requires multiple mice to work

together. It is likely that not every participant invests the same amount of energy in this cooperative action (Serra et al. 2012) but completing a structure like this in such a short time by working alone is improbable. Thus, the mounds built by a group of individuals can be considered as a ‘public achievement’ from which all individuals gain benefit.

The closest relatives of the mound-building mice are all endemic to warmer climates. The Macedonian mouse occurs in the south Balkans, Asia Minor and the Middle East, the Algerian mouse inhabits south-western Europe and the western Mediterranean coast of Africa. Mitochondrial DNA and nuclear gene sequences suggest that the North Indian subcontinent as the evolutionary origin and radiation of the house mouse (Boursot et al. 1996; Din et al. 1996). The commensalism, which resulted in the unprecedented colonization success of this species probably occurred in the Fertile Crescent (Auffray et al. 1988). It is probable that the mound-building mouse originated from South-east Europe and its successful overwintering strategy with the related behavioral traits made spreading further north possible.

DELAYED DISPERSAL ENABLES COMMUNAL OVERWINTERING

Natal dispersal plays a fundamental role in demographic processes and in the formation of social groups (Gaines and McClenaghan 1980; Perrin and Goudet 2001). Polygynous mammals usually show a male-biased dispersal, while both sexes disperse or dispersal is female-biased in monogamous species (Dobson 1982; Favre et al. 1997; Greenwood 1980; Perrin and Mazalov 2000; Wolff 1993). In addition, other life history traits also have effects on natal and adult dispersal. Ecological constraints can lead to seasonal or life stage dependent aggregation, like the lack of breeding places enforces the development of helping or alloparental behavior (Hatchwell and Komdeur 2000; Kokko and Lundberg 2001; König 1997). The polygynous mating system and ecology of the house mouse implies fast maturation and male biased dispersal (Gerlach 1990, 1996; Lidicker 1976; van Zegeren 1980). Fathers expel their mature sons, or the dominant sibling takes his place. Young males can choose between two strategies: mature early, grow fast and fight for the dominant position, or wait until they reach a better physiological condition and find themselves a suitable habitat. Females, on the other hand, can tolerate their siblings, and under certain ecological conditions might breed together (Dobson and Baudoin 2002; Dobson et al. 2000; König 1993; Manning et al. 1995; Sayler and Salmon 1969). Hence, their

motivation for dispersal is reduced. However, since breeding success in such female groups is often biased, dispersal can be advantageous for subordinates.

In a comparative test of natal dispersal, where only the young animals were used, I found that mice may show dispersal even without the influence of their parents. The result on the dispersal pattern of the house mouse is in accordance with previous studies. House mouse males disperse earlier and in higher ratio than females. However, mound building mice disperse later and do not show sex bias in natal dispersal. The observed dispersal pattern in the mound-building mouse is in agreement both with its socially monogamous mating system (Baudoïn et al. 2005; Patris et al. 2002) and their communal overwintering strategy. Presumably, the young mice delay their dispersal due to the need of the cooperative mound building. The use of mounds enhance the probability of surviving through winter, thus the benefit of the mounds during the winter exceeds the costs of the delayed reproduction. Since limited dispersal (population viscosity) keeps relatives together (Hamilton 1964), altruism directed toward all neighbors will be favored as neighbors tend to be relatives. This form of kin selection has been suggested to be important for the production of public goods (Brown 1999). In the case of the mound-building mouse, not leaving the family group and build the overwintering mounds with their siblings seems favorable. Next spring, when the mice leave their mounds, to find pairs and start to reproduce, males and females disperse simultaneously. The lack of sexual dimorphism in their dispersal supports their monogamous mating system.

SELECTIVE TOLERANCE KEEPS GROUPS TOGETHER

The intensity and direction of agonistic behavior, the overall or selective tolerance among individuals is mainly under the influence of ecological conditions and in direct connection with sociality, the existence of social groups, and the type of the breeding system (Armitage 1981; Crowcroft and Rowe 1963; Getz and Carter 1980; Getz et al. 1981; Insel et al. 1995; Livoreil et al. 1993; Patris et al. 2002; Sachser et al. 1999; Shapiro et al. 1986). Aggression have a major role in shaping the social structure of a population and the spatial distribution of individuals through territorial behavior (Grant 1972). In my comparative study on social and aggressive behavior of the mound-building mouse and the house mouse, I found distinct differences between this two species. Moreover, the observed characteristics are parallel to their social systems.

The average level of aggression elevated with age in both species. Both sexes of the mound-building mouse were tolerant with their sibling (with whom they were raised together) while the house mouse males were not. House mouse females did not attack their littermates either. When faced an unfamiliar opponent both sexes of the mound-building mouse were more aggressive than the house mouse.

The social systems of this two mouse species are different, and it is reflected in their social behavior. The house mouse is polygynous, maintaining social group territoriality (Bronson 1979; Brown 1953; Crowcroft and Rowe 1963; Lidicker 1976). Males compete for females and develop dominance-submission relationship inside the social group (Bronson 1979; Crowcroft and Rowe 1963; Lidicker 1976; Reimer and Petras 1967). Cooperation can be observed among females (Dobson and Baudoin 2002; Dobson et al. 2000; König 1993; Manning et al. 1995; Sayler and Salmon 1969). Hence, males are more agonistic than females, while females tolerate each other in certain circumstances. The mound-building mouse are monogamous and juveniles overwinter in mixed sex groups (Baudoin et al. 2005; Murariu 1981; Naumov 1940; Patris et al. 2002; Sokolov et al. 1998). The observed tolerance among siblings and the elevated level of aggression against unfamiliar is essential in the maintaining of the communally overwintering groups, as well as protecting their mounds from individuals who did not participated in the building. Further genetic and behavioral studies would be necessary to clarify whether the groups are truly based on kinship under natural conditions.

Chapter IV.

OVERALL CONCLUSIONS

In this thesis I demonstrated, that the mounds of the mound-building mouse are integral parts of their overwintering behaviour. With field studies and experiments under seminatural conditions I demonstrated that the mounds have thermal and water insulating capacities which may contribute to the successful overwintering of this species. Contrary to the common belief, I found no evidence that the mice consume the hoarded plant material. The use of mounds enhance the probability of surviving through winter, thus the benefit of the mounds during the winter exceeds the costs of the delayed reproduction.

The aggressive behaviour and natal dispersal of the mound-building mouse reflect the needs of the communal overwintering and the monogamous mating system. The observed tolerance among siblings and the elevated level of aggression against unfamiliar is essential in the maintaining of the communally overwintering groups. Limited dispersal keeps siblings together, making cooperatively building the mounds easier and evolutionary more profitable, since the members of the groups are close relatives.

I demonstrated that:

- That the morphology of the mound do not but the location of the nest chamber depends on soil characteristics
- During their overwintering the mound-building mice do not consume the collected vegetable material
- The mound alters the temperature profile and water content of the soil beneath
- The collected plant material plays an important role in keeping the nest chamber dry
- During their overwintering the mortality rate of the mound-building mouse is low

- Both sexes of the mound-building mouse shows elevated level of aggression compared to the house mouse, but remains tolerant toward their familiar sibling even after long separation
- The mound-building disperse at a later age than house mouse
- There was no sexual dimorphism in the natal dispersal of the mound-building mouse
- Weight at weaning do not affect the age of dispersal of the mound-building mouse

The unique overwintering strategy and the monogamous mating system make the mound-building mouse a good model for ethological studies. Revealing the genetic structure of the overwintering social groups and comparative experiments with more related species would provide valuable information on the behavioral bases of cooperation and the ecological constraints shaping social systems.

REFERENCES

- Aars J, Ims RA (2002) Intrinsic and climatic determinants of population demography: The winter dynamics of tundra voles. *Ecology* 83 (12):3449-3456.
- Adams DB (1980) Motivational systems of agonistic behavior in muroid rodents: A comparative review and neural model. *Aggressive Behavior* 6 (4):295-346.
- Ågren G, Zhou Q, Zhong W (1989) Territoriality, cooperation and resource priority: hoarding in the Mongolian gerbil, *Meriones unguiculatus*. *Animal Behaviour* 37 (1):28-32.
- Aldhous P (1989) The effects of individual cross-fostering on the development of intrasexual kin discrimination in male laboratory mice, *Mus musculus*. *Animal Behaviour* 37:741-750.
- Alexander RD (1974) The Evolution of Social Behavior. *Annual Review of Ecology and Systematics* 5:325-383.
- Allaine D, Brondex F, Graziani L, Coulon J, Till-Bottraud I (2000) Male-biased sex ratio in litters of Alpine marmots supports the helper repayment hypothesis. *Behavioral Ecology* 11 (5):507-514.
- Armitage KB (1981) Sociality as a life-history tactic of ground-squirrels. *Oecologia* 48 (1):36-49.
- Auffray JC, Tchernov E, Nevo E (1988) Origine du commensalisme de la souris domestique (*Mus musculus domesticus*). *Comptes rendus de l'Académie des sciences* 307 (9):517-522.
- Axelrod R, Hamilton WD (1981) The Evolution of Cooperation. *Science* 211 (4489):1390-1396.
- Banks PB, Dickman CR (2000) Effects of winter food supplementation on reproduction, body mass, and numbers of small mammals in montane Australia. *Canadian Journal of Zoology* 78 (10):1775-1783.
- Barclay RMR, Lausen CL, Hollis L (2001) What's hot and what's not: defining torpor in free-ranging birds and mammals. *Canadian Journal of Zoology* 79 (10):1885-1890.
- Barnard CJ, Fitzsimons J (1988) Kin recognition and mate choice in mice: the effects of kinship, familiarity and social interference on intersexual interaction. *Animal Behaviour* 36 (4):1078-1090.
- Baudoin C, Busquet N, Dobson FS, Gheusi G, Feron C, Durand JL, Heth G, Patris B, Todrank J (2005) Male-female associations and female olfactory neurogenesis with pair bonding in *Mus spicilegus*. *Biological Journal of the Linnean Society* 84 (3):323-334.
- Belthoff JR, Dufty AMJ (1998) Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. *Animal Behaviour* 55 (2):405-415.
- Benz JJ (1993) Food-elicited vocalizations in golden lion tamarins: design features for representational communication. *Animal Behaviour* 45 (3):443.

- Bernstein IS (1981) Dominance - the baby and the bathwater. *Behavioral and Brain Sciences* 4 (3):419-429.
- Berry RJ, Jakobson ME, Triggs GS (1973) Survival in wild-living mice. *Mammal Review* 3 (2):46-57.
- Bester-Meredith JK, Young LJ, Marler CA (1999) Species differences in paternal behavior and aggression in *peromyscus* and their associations with vasopressin immunoreactivity and receptors. *Hormones and Behavior* 36 (1):25-38.
- Bethge P, Munks S, Otley H, Nicol S (2004) Platypus burrow temperatures at a subalpine tasmanian lake. *Proceedings of the Linnean Society of New South Wales* 125:273-227.
- Bihari Z (2004a) A güzüegér (*Mus spicilegus*) életmódjának sajátosságai és mezőgazdasági jelentősége. *Növényvédelem* 40:245-250.
- Bihari Z (2004b) A güzüegér magyarországi elterjedése és építő tevékenységének jellemzői. *Vadbiológia* 10:107-114.
- Blaustein AR (1983) Kin recognition mechanisms: phenotypic matching or recognition alleles? *The American Naturalist* 121 (5):749-754.
- Bonhomme F, Catalan J, Britton-Davidian J, Chapman VM, Moriwaki K, Nevo E, Thaler L (1984) Biochemical diversity and evolution in the genus *Mus*. *Biochemical Genetics* 22 (3):275-303.
- Boursot P, Auffray JC, Britton-Davidian J, Bonhomme F (1993) The evolution of house mice. *Annual Review of Ecology and Systematics* 24 (1):119-152.
- Boursot P, Din W, Anand R, Darviche D, Dod B, Von Deimling F, Talwar G, Bonhomme F (1996) Origin and radiation of the house mouse: mitochondrial DNA phylogeny. *Journal of Evolutionary Biology* 9 (4):391-415.
- Bowler DE, Benton TG (2005) Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews* 80 (2):205-225.
- Bozikova M, Hlavac P (2005) Thermophysical parameters of chosen biological materials. Paper presented at the BioPhys Spring, 4th International Conference of Young Scientists, Lublin, Poland,
- Bradshaw RH (1992) The spring decline in a population of field voles, *Microtus agrestis*, and the onset of severe winter weather. *Mammalia* 56 (2):292-295.
- Brain PF, Parmigiani S (1990) Variation in aggressiveness in house mouse populations. *Biological Journal of the Linnean Society* 41 (1 3):257-269.
- Broadbrooks HE (1965) Ecology and distribution of the pikas of Washington and Alaska. *American Midland Naturalist* 61:411-416.
- Bronson FH (1979) The reproductive ecology of the House mouse. *The Quarterly Review of Biology* 54 (3):265-299.

- Bronson FH (1985) Mammalian reproduction: an ecological perspective. *Biology of Reproduction* 32 (1):1-26.
- Bronson FH, Perrigo G (1987) Seasonal regulation of reproduction in muroid rodents. *American Zoologist* 27 (3):929-940.
- Brown CR, Brown MB (1986) Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). *Ecology* 67 (5):1206-1218.
- Brown JL (1987) Helping and communal breeding in birds. Princeton University Press, Princeton
- Brown RZ (1953) Social behavior, reproduction, and population changes in the house mouse (*Mus musculus* L.). *Ecological Monographs* 23 (3):218-240.
- Brown SP (1999) Cooperation and conflict in host-manipulating parasites. *Proceedings of the Royal Society of London Series B: Biological Sciences* 266 (1431):1899-1904.
- Canady A, Mosansky L, Stanko M (2009) First knowledge of winter ecology of the Mound-building mouse (*Mus spicilegus* Petenyi, 1882) from Slovakia. *Acta Zoologica Bulgarica* 61 (1):79-86.
- Canals M, Rosenmann M, Bozinovic F (1989) Energetics and geometry of huddling in small mammals. *Journal of Theoretical Biology* 141 (2):181-189.
- Carranza J (ed) (2000) Environmental effects on the evolution of mating systems in endotherms. *Vertebrate Mating Systems*. World Scientific Pub Co Inc, Erice, Italy
- Casey TM (1981) Nest insulation: Energy savings to brown lemmings using a winter nest. *Oecologia* 50 (2):199-204.
- Charnov EL, Finerty JP (1980) Vole population cycles: A case for kin-selection? *Oecologia* 45 (1):1-2.
- Chevret P, Veyrunes F, Britton-Davidian J (2005) Molecular phylogeny of the genus '*Mus*' (Rodentia: Murinae) based on mitochondrial and nuclear data. *Biological Journal of the Linnean Society* 84 (3):417-427.
- Clobert J, Ims RA, Rousset F (2004) Causes, mechanisms, and consequences of dispersal. In: Hanski I, Gaggiotti OE (eds) *Ecology, Genetics, and Evolution of Metapopulations*. Elsevier Academic Press, London, pp 307-335
- Clutton-Brock TH (1989) Mammalian mating systems. *Proceedings of the Royal Society of London Series B: Biological Sciences* 236 (1285):339-372.
- Clutton-Brock TH (2002) Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science* 296 (5565):69-72.
- Contreras LC (1984) Bioenergetics of Huddling - Test of a Psycho-Physiological Hypothesis. *Journal of Mammalogy* 65 (2):256-262.
- Creel SR, Creel NM (1991) Energetics, reproductive suppression and obligate communal breeding in carnivores. *Behavioral Ecology and Sociobiology* 28 (4):263-270.

- Crowcroft P, Rowe FP (1963) Social organization and territorial behaviour in the wild house mouse (*Mus musculus* L.). Proceedings of the Zoological Society of London 140 (3):517-531.
- Davies NB, Krebs JR, West SA (2012) An introduction to behavioural ecology. Wiley-Blackwell, Oxford
- Davis LS (1982) Sibling recognition in Richardson's ground squirrels (*Spermophilus richardsonii*). Behavioral Ecology and Sociobiology 11 (1):65-70.
- Dearing MD (1997) The function of haypiles of pikas (*Ochotona princeps*). Journal of Mammalogy 78 (4):1156-1163.
- Demeter A, Rácz G, Csorba G (1995) Identification of house mice (*Mus musculus*) and mound-building mice (*Mus spicilegus*) using distance and landmark data. In: Marcus LF, Corti M, Loy A, Naylor G, Slice DE (eds) Advances in Morphometrics. Plenum Press, New York, pp 359-369
- Dewsbury DA (1988) Kinship, familiarity, aggression, and dominance in Deer mice (*Peromyscus maniculatus*) in seminatural enclosures. Journal of Comparative Psychology 102 (2):124-128.
- Dieckmann U, O'Hara B, Weisser W (1998) The evolutionary ecology of dispersal. Working Papers.
- Din W, Anand R, Boursot P, Darviche D, Dod B, Jouvin-Marche E, Orth A, Talwar GP, Cazenave PA, Bonhomme F (1996) Origin and radiation of the house mouse: clues from nuclear genes. Journal of Evolutionary Biology 9 (5):519-539.
- Dingle H (1996) Migration: The biology of life on the move. Oxford University Press, New York
- Dobson FS (1982) Competition for mates and predominant juvenile male dispersal in mammals. Animal Behaviour 30 (Nov):1183-1192.
- Dobson FS, Jones WT (1985) Multiple causes of dispersal. The American Naturalist 126 (6):855-858.
- Dobson FS, Jacquot C, Baudoin C (2000) An experimental test of kin association in the house mouse. Canadian Journal of Zoology 78 (10):1806-1812.
- Dobson FS, Baudoin C (2002) Experimental tests of spatial association and kinship in monogamous mice (*Mus spicilegus*) and polygynous mice (*Mus musculus domesticus*). Canadian Journal of Zoology 80 (6):980-986.
- Drickamer LC (1975) Female mouse maturation: relative importance of social factors and daylength. Journal of Reproduction and Fertility 44 (1):147.
- Drickamer LC (1982) Delay and acceleration of puberty in female mice by urinary chemosignals from other females. Developmental Psychobiology 15 (5):433-445.
- Drickamer LC (1989) Intermittent stimulation and delay of puberty by urinary chemosignals and social contact in wild stock female house mice (*Mus domesticus*). Behavioural Processes 20 (1-3):75-83.
- Dudley D (1974) Paternal behavior in the california mouse, *Peromyscus californicus*. Behavioral Biology 11 (2):247-252.

- Dufty AM, Belthoff JR (2001) Proximate mechanisms of natal dispersal: the role of body condition and hormones. In: Colbert J, Danchin E, Dhondt AA, Nichols JD (eds) Dispersal. Oxford University Press, Oxford, pp 217 -229
- Dugatkin LA (1997) Cooperation among animals: an evolutionary perspective. Oxford University Press, New York
- Ebensperger LA, Bozinovic F (2000) Energetics and burrowing behaviour in the semifossorial degu *Octodon degus* (Rodentia: Octodontidae). *Journal of Zoology* 252 (02):179-186.
- Emlen ST, Oring LW (1977) Ecology, sexual selection, and evolution of mating systems. *Science* 197 (4300):215-223.
- Endler J (1977) Geographic variation, speciation, and clines. Princeton University Press, Princeton
- Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal *Crocodyrus russula*: Evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London Series B-Biological Sciences* 264 (1378):127-132.
- Feron C, Gheusi G (2003) Social regulation of reproduction in the female mound-builder mouse (*Mus spicilegus*). *Physiology & Behavior* 78 (4-5):717-722.
- Festetics A (1961) Ährenmaushügel in Österreich. *Zeitschrift für Säugetierkunde* 26:112-125.
- Formozov AN (1966) Adaptive modifications of behavior in mammals of the eurasian steppes. *Journal of Mammalogy* 47 (2):208-223.
- Frynta D, Slábová M, Váchová H, Volfová R, Munclinger P (2005) Aggression and commensalism in house mouse: a comparative study across Europe and the Near East. *Aggressive Behavior* 31:283-293.
- Gadgil M (1971) Dispersal: population consequences and evolution. *Ecology* 52 (2):253-261.
- Gaines MS, McClenaghan LR (1980) Dispersal in small mammals. *Annual Review of Ecology and Systematics* 11 (1):163-196.
- Galef BG (1991) Information-centers of Norway rats - sites for information exchange and information parasitism. *Animal Behaviour* 41:295-301.
- Ganem G, Searle JB (1996) Behavioural discrimination among chromosomal races of the house mouse (*Mus musculus domesticus*). *Journal of Evolutionary Biology* 9 (6):817-830.
- Ganem G, Ginane C, Cile, Ostrowski M-F, Orth A (2005) Assessment of mate preference in the house mouse with reference to investigations on assortative mating. *Biological Journal of the Linnean Society* 84:461-471.
- Gannon WL, Sikes RS (2007) Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 88 (3):809-823.

- Garza JC, Dallas J, Duryadi D, Gerasimov S, Croset H, Boursot P (1997) Social structure of the mound-building mouse *Mus spicilegus* revealed by genetic analysis with microsatellites. *Molecular Ecology* 6 (11):1009-1017.
- Gavish L, Hofmann JE, Getz LL (1984) Sibling recognition in the Prairie vole, *Microtus ochrogaster*. *Animal Behaviour* 32 (May):362-366.
- Gedeon CI, Markó G, Németh I, Nyitrai V, Altbäcker V (2010) Nest material selection affects nest insulation quality for the European ground squirrel (*Spermophilus citellus*). *Journal of Mammalogy* 91 (3):636-641.
- Geiser F (1988) Reduction of metabolism during hibernation and daily torpor in mammals and birds - temperature effect or physiological inhibition. *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology* 158 (1):25-37.
- Geiser F (1998) Evolution of daily torpor and hibernation in birds and mammals: importance of body size. *Clinical and Experimental Pharmacology & Physiology* 25 (9):736-739.
- Gerlach G (1990) Dispersal mechanisms in a captive wild house mouse population (*Mus domesticus* Rutty). *Biological Journal of the Linnean Society* 41 (1-3):271-277.
- Gerlach G (1996) Emigration mechanisms in feral house mice: a laboratory investigation of the influence of social structure, population density, and aggression. *Behavioral Ecology and Sociobiology* 39 (3):159-170.
- Getz LL, Carter CS (1980) Social organization in *Microtus ochrogaster* populations. *Biologist* 62 (1-4):56-69.
- Getz LL, Carter CS, Gavish L (1981) The mating system of the Prairie vole (*Microtus ochrogaster*): field and laboratory evidence for pair-bonding. *Behavioral Ecology and Sociobiology* 8 (3):189-194.
- Gipps JHW, Flynn MP, Gurnell J, Healing TD (1985) The spring decline in populations of the bank vole, *Clethrionomys glareolus*, and the role of female density. *Journal of Animal Ecology* 54 (2):351-358.
- Glaser H, Lustick S (1975) Energetics and nesting-behavior of northern white-footed mouse, *Peromyscus leucopus noveboracensis*. *Physiological Zoology* 48 (2):105-113.
- Gomendio M, Martin-Coello J, Crespo C, Magana C, Roldan ERS (2006) Sperm competition enhances functional capacity of mammalian spermatozoa. *Proceedings of the National Academy of Sciences of the United States of America* 103 (41):15113-15117.
- Gouat P, Feron C, Demouron S (2003a) Seasonal reproduction and delayed sexual maturity in mound-building mice *Mus spicilegus*. *Reproduction Fertility and Development* 15 (3):187-195.

- Gouat P, Katona K, Poteaux C (2003b) Is the socio-spatial distribution of mound-building mice, *Mus spicilegus*, compatible with a monogamous mating system? *Mammalia* 67 (1):15-24.
- Gouat P, Feron C (2005) Deficit in reproduction in polygynously mated females of the monogamous mound-building mouse *Mus spicilegus*. *Reproduction Fertility and Development* 17 (6):617-623.
- Grant EC, Mackintosh JH (1963) A comparison of the social postures of some common laboratory rodents. *Behaviour* 21:246-259
- Grant PR (1972) Interspecific competition among rodents. *Annual Review of Ecology and Systematics* 3:79-106.
- Grau HJ (1982) Kin recognition in White-footed deer mice (*Peromyscus leucopus*). *Animal Behaviour* 30 (2):497-505.
- Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* 28 (4):1140-1162.
- Greenwood PJ, Harvey PH (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics* 13 (1):1-21.
- Griffin AS, West SA (2002) Kin selection: fact and fiction. *Trends in Ecology & Evolution* 17 (1):15-21.
- Griffin AS, West SA (2003) Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science* 302 (5645):634-636.
- Grubbauer P, Hoi H (1996) Female penduline tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success. *Ecoscience* 3 (3):274-279.
- Gubernick DJ, Alberts JR (1987) The biparental care system of the California mouse, *Peromyscus californicus*. *Journal of Comparative Psychology* 101:169-177.
- Hager R, Johnstone RA (2007) Maternal and offspring effects influence provisioning to mixed litters of own and alien young in mice. *Animal Behaviour* 74:1039-1045.
- Halpin ZT, Hoffman MD (1987) Sibling recognition in the white-footed mouse, *Peromyscus leucopus* - association or phenotype matching. *Animal Behaviour* 35:563-570.
- Hamilton WD (1964) The genetical evolution of social behavior. *Journal of Theoretical Biology* 7:1-52.
- Hardin G (1968) The tragedy of the commons. *Science* 162:124-148.
- Hatchwell BJ, Komdeur J (2000) Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour* 59:1079-1086.
- Hayes JP, Speakman JR, Racey PA (1992) The contributions of local heating and reducing exposed surface area to the energetic benefits of huddling by short-tailed field voles (*Microtus agrestis*). *Physiological Zoology* 65 (4):742-762.

- Heldmaier G, Ortmann S, Elvert R (2004) Natural hypometabolism during hibernation and daily torpor in mammals. *Respiratory Physiology & Neurobiology* 141 (3):317-329.
- Hilton GM, Hansell MH, Ruxton GD, Reid JM, Monaghan P, Brittingham M (2004) Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. *The Auk* 121 (3):777-787.
- Holekamp KE (1984) Natal dispersal in Belding ground-squirrels (*Spermophilus beldingi*). *Behavioral Ecology and Sociobiology* 16 (1):21-30.
- Holmes WG, Sherman PW (1982) The ontogeny of kin recognition in two species of ground squirrels. *American Zoologist* 22 (3):491-517.
- Holmes WG (1984) Sibling recognition in thirteen-lined ground squirrels: effects of genetic relatedness, rearing association, and olfaction. *Behavioral Ecology and Sociobiology* 14 (3):225-233.
- Hölzl M, Hoi H, Darolova A, Krištofik J, Penna DJ (2009) Why do the mounds of *Mus spicilegus* vary so much in size and composition? *Mammalian Biology* 74 (4):308-314
- Hölzl M, Hoi H, Darolova A, Krištofik J (2011a) Insulation capacity of litter mounds built by *Mus spicilegus*: physical and thermal characteristics of building material and the role of mound size. *Ethology Ecology & Evolution* 23 (1):49-59.
- Hölzl M, Krištofik J, Darolová A, Hoi H (2011b) Food preferences and mound-building behaviour of the mound-building mice *Mus spicilegus*. *Naturwissenschaften* 98 (10):863-870.
- Houston AI, McNamara JM (1993) A theoretical investigation of the fat reserves and mortality levels of small birds in winter. *Ornis Scandinavica* 24 (3):205-219.
- Howard WE (1960) Innate and environmental dispersal of individual vertebrates. *American Midland Naturalist* 63:152-161.
- Hudson R, Trillmich F (2008) Sibling competition and cooperation in mammals: challenges, developments and prospects. *Behavioral Ecology and Sociobiology* 62 (3):299-307.
- Humphries MM, Thomas DW, Kramer DL (2003) The role of energy availability in mammalian hibernation: A cost-benefit approach. *Physiological and Biochemical Zoology* 76 (2):165-179.
- Insel TR, Preston S, Winslow JT (1995) Mating in the monogamous male: behavioral consequences. *Physiology & Behavior* 57 (4):615-627.
- IUCN Red List of Threatened Species (2012) International Union for Conservation of Nature. <http://www.iucnredlist.org/>. Accessed August, 2012
- Jackson DM, Trayhurn P, Speakman JR (2001) Associations between energetics and over-winter survival in the short-tailed field vole *Microtus agrestis*. *Journal of Animal Ecology* 70 (4):633-640.

- Janský L (1973) Non-shivering thermogenesis and its thermoregulatory significance. *Biological Reviews* 48 (1):85-132.
- Johnson ML, Gaines MS (1990) Evolution of dispersal: theoretical models and empirical tests using birds and mammals. *Annual Review of Ecology and Systematics* 21:449-480.
- Katona K, Altbäcker V (2002) Diet estimation by faeces analysis: sampling optimisation for the European hare. *Folia Zoologica* 51 (1):11-15.
- Kay FR, Whitford WG (1978) The burrow environment of the banner-tailed kangaroo rat, *Dipodomys spectabilis*, in southcentral New Mexico. *American Midland Naturalist* 99 (2):270-279.
- Kenagy GJ (1973) Daily and seasonal patterns of activity and energetics in a heteromyid rodent community. *Ecology* 54 (6):1201-1219.
- Kleiman DG (1977) Monogamy in mammals. *The Quarterly Review of Biology* 52 (1):39-69.
- Kokko H, Lundberg P (2001) Dispersal, migration, and offspring retention in saturated habitats. *The American Naturalist* 157 (2):188-202.
- Komers PE, Brotherton PNM (1997) Female space use is the best predictor of monogamy in mammals. *Proceedings of the Royal Society of London Series B: Biological Sciences* 264 (1386):1261-1270.
- König B (1993) Maternal investment of communally nursing female house mice (*Mus musculus domesticus*). *Behavioural Processes* 30:61-74.
- König B (1994) Fitness effects of communal rearing in house mice: The role of relatedness versus familiarity. *Animal Behaviour* 48:1449-1457.
- König B (1997) Cooperative care of young in mammals. *Naturwissenschaften* 84 (3):95-104.
- Körtner G, Geiser F (2000) The temporal organization of daily torpor and hibernation: circadian and circannual rhythms. *Chronobiology International* 17 (2):103-128.
- Korytko AI, Vessey SH (1991) Agonistic and spacing behaviour in white-footed mice, *Peromyscus leucopus*. *Animal Behaviour* 42 (6):913-919.
- Krackow S (2003) Motivational and heritable determinants of dispersal latency in wild male house mice (*Mus musculus musculus*). *Ethology* 109 (8):671-689.
- Krause J, Ruxton GD (2002) *Living in groups*. Oxford University Press, Oxford
- Lamprecht I, Schmolz E (2004) Thermal investigations of some bird nests. *Thermochimica Acta* 415 (1-2):141-148.
- Lidicker WZ (1976) Social behaviour and density regulation in house mice living in large enclosures. *Journal of Animal Ecology* 45:677-697.
- Lima SL (1989) Iterated prisoner's dilemma: an approach to evolutionarily stable cooperation. *The American Naturalist* 134 (5):828-834.

- Lindstrom E (1986) Territory inheritance and the evolution of group-living in carnivores. *Animal Behaviour* 34:1825-1835.
- Livoreil B, Gouat P, Baudoin C (1993) A comparative study of social behaviour of two sympatric ground squirrels (*Spermophilus pilosoma*, *S. mexicanus*). *Ethology* 93 (3):236-246.
- Lovegrove BG, Raman J, Perrin MR (2001) Heterothermy in elephant shrews, *Elephantulus spp.* (Macroscelidea): daily torpor or hibernation? *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology* 171 (1):1-10.
- Lundrigan BL, Jansa SA, Tucker PK (2002) Phylogenetic relationships in the genus *Mus*, based on paternally, maternally, and biparentally inherited characters. *Systematic Biology* 51 (3):410-431.
- Manning CJ, Wakeland EK, Potts WK (1992) Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature* 360:581 - 583.
- Manning CJ, Dewsbury DA, Wakeland EK, Potts WK (1995) Communal nesting and communal nursing in house mice, *Mus musculus domesticus*. *Animal Behaviour* 50:741-751.
- Mateo JM (2003) Kin recognition in ground squirrels and other rodents. *Journal of Mammalogy* 84 (4):1163-1181.
- Mátrai K, Altbácker V, Hahn I (1998) Seasonal diet of rabbits and their browsing effect on juniper in Bugac Juniper forest (Hungary). *Acta Theriologica* 43 (1):107-112.
- Maynard Smith J (1983) Game theory and the evolution of cooperation. In: Bendall DS (ed) *Evolution from molecules to men* Cambridge University Press, Cambridge, pp 445-456
- McCafferty DJ, Moncrieff JB, Taylor IR (2003) Winter microclimate of field voles (*Microtus agrestis*) in SW Scotland. *Journal of Thermal Biology* 28 (5):397-401.
- McGowan A, Sharp SP, Hatchwell BJ (2004) The structure and function of nests of long-tailed tits, *Aegithalos caudatus*. *Functional Ecology* 18 (4):578-583.
- McGuire B, Getz LL, Hofmann JE, Pizzuto T, Frase B (1993) Natal dispersal and philopatry in prairie voles (*Microtus ochrogaster*) in relation to population density, season, and natal social environment. *Behavioral Ecology and Sociobiology* 32 (5):293-302.
- McNab BK (2008) An analysis of the factors that influence the level and scaling of mammalian BMR. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 151 (1):5-28.
- Mertens JAL (1977) Thermal conditions for successful breeding in great tits (*Parus major L.*). *Oecologia* 28 (1):31-56.
- Metzgar LH (1971) Behavioral population regulation in the woodmouse, *Peromyscus leucopus*. *American Midland Naturalist* 86:434-448.

- Milishnikov AN, Rafiev AN, Muntianu AI (1998) Genotypic variability in populations of moundbuilder mice *Mus spicilegus* Pet., 1882, at different life-cycle stages. Russian Journal of Genetics 34:785-790.
- Millesi E, Hoffmann IE, Steurer S, Metwaly M, Dittami JP (2002) Vernal changes in the behavioral and endocrine responses to GnRH application in male European ground squirrels. Hormones and Behavior 41 (1):51-58.
- Munclinger P, Frynta D (2000) Social interactions within and between two distant populations of house mouse. Folia Zoologica 49 (1):1-6.
- Muntyanu AI (1990) Ecological features of an overwintering population of the hillock mouse (*Mus hortulanus* Nordm.) in the south-west of the U.S.S.R. . Biological Journal of the Linnean Society 41:73-82.
- Murariu D (1981) La présence de *Mus musculus* spicilegus Petenyi, 1882 dans le Delta du Danube accompagné de son 'parasite' *Apodemus agrarius* (Pall., 1771). Travaux Du Museum d'Histoire Naturelle 'Grigore Antipa' 23:297-304.
- Natori Y, Porter WP (2007) Model of Japanese serow (*Capricornis crispus*) energetics predicts distribution on Honshu, Japan. Ecological Applications: a publication of the Ecological Society of America 17 (5):1441-1459.
- Naumov NP (1940) Ecology of the mound-builder mouse *Mus musculus hortolanus*. Works Institute Evolutionary Morphology USSR 3:33-76.
- Németh I, Nyitrai V, Altbäcker V (2009) Ambient temperature and annual timing affect torpor bouts and euthermic phases of European ground squirrels (*Spermophilus citellus*). Canadian Journal of Zoology 87 (3):204-210.
- Nowak M, Sigmund K (1993) A strategy of win-stay, lose-shift that outperforms Tit-for-Tat in the Prisoners-dilemma game. Nature 364 (6432):56-58.
- Nowak MA, May RM (1992) Evolutionary games and spatial chaos. Nature 359 (6398):826-829.
- Nunes S, Holekamp KE (1996) Mass and fat influence the timing of natal dispersal in Belding's ground squirrels. Journal of Mammalogy 77 (3):807-817.
- Nunes S, Ha CDT, Garrett PJ, Mueke EM, Smale L, Holekamp KE (1998) Body fat and time of year interact to mediate dispersal behaviour in ground squirrels. Animal Behaviour 55:605-614.
- O'Riain MJ, Jarvis JUM, Faulkes CG (1996) A dispersive morph in the naked mole-rat. Nature 380 (6575):619-621.
- Oliveras D, Novak M (1986) A comparison of paternal behavior in the meadow vole *Microtus pennsylvanicus*, the pine vole *M. pinetorum* and the prairie vole *M. ochrogaster*. Animal Behaviour 34:519-526.

- Orsini P, Bonhomme F, Britton-Davidian J, Croset H, Gerasimov S, Thaler L (1983) Le complexe d'espèces du genre *Mus* en Europe Centrale et Orientale. II critères d'identification, répartition et caractéristiques écologiques. *Zeitschrift für Säugetierkunde* 48:86-95.
- Packer C, Lewis S, Pusey A (1992) A comparative analysis of non-offspring nursing. *Animal Behaviour* 43 (2):265-281.
- Palanza P, D. Mainardi LR, Brain PF, Parmigiani S (1996) Male and female competitive strategies of wild house mice pairs (*Mus musculus domesticus*) confronted with intruders of different sex and age in artificial territories. *Behaviour* 133:863-882.
- Parmigiani S (1984) Communal nursing and maternal aggression in female mice (*Mus musculus domesticus*). *Aggressive Behavior* 10 (2):166-166.
- Parmigiani S, Palanza P, Brain PF (1989) Intraspecific maternal aggression in the house mouse (*Mus domesticus*): A counterstrategy to infanticide by male? *Ethology Ecology & Evolution* 1 (4):341-352.
- Parmigiani S, Palanza P (1994) Social behavior of wild house mice: the role of the neglected female. . *Bollettino di Zoologia* 61:47-48.
- Pásztor L, Szabó J, Várallyay G (1996) Digging deep for global soil and terrain data. *GIS Europe* 5 (8):32-34.
- Patris B, Baudoin C (1998) Female sexual preferences differ in *Mus spicilegus* and *Mus musculus domesticus*: the role of familiarization and sexual experience. *Animal Behaviour* 56:1465-1470.
- Patris B, Baudoin C (2000) A comparative study of parental care between two rodent species: implications for the mating system of the mound-building mouse *Mus spicilegus*. *Behavioural Processes* 51 (1-3):35-43.
- Patris B, Gouat P, Jacquot C, Christophe N, Baudoin C (2002) Agonistic and sociable behaviors in the mound-building mice, *Mus spicilegus*: A comparative study with *Mus musculus domesticus*. *Aggressive Behavior* 28 (1):75-84.
- Pellis SM, Pellis VC, Pierce J. D. Jr, Dewsbury DA (1992) Disentangling the contribution of the attacker from that of defender in the differences in the intraspecific fighting in two species of voles. *Aggressive Behavior* 18 (6):425-435.
- Perrin N, Mazalov V (2000) Local competition, inbreeding, and the evolution of sex biased dispersal. *The American Naturalist* 155:116-127.
- Perrin N, Goudet J (2001) Inbreeding, kinship, and the evolution of natal dispersal. In: Clobert J, Danchin E, Dhondt AA, Nichols JD (eds) *Dispersal: individual, population and community*. Oxford University Press, Oxford, pp 127-146

- Perrin N, Lehmann L (2001) Is sociality driven by the costs of dispersal or the benefits of philopatry? A role for kin discrimination mechanisms. *The American Naturalist* 158 (5):471-483.
- Pinowski J, Hamanb A, Jerzak L, Pinowskaa B, Barkowskad M, Grodzkie A, Hamanf K (2006) The thermal properties of some nests of the Eurasian tree sparrow, *Passer montanus*. *Journal of Thermal Biology* 31 (7):573-581.
- Pisareva ME (1948) Contribution to ecology and systematic of the mound-building mouse. *Annals of the Biology Faculty of Dniepropetrovsk State University* 32:68-71.
- Pitt JA, Larivière S, Messier F (2008) Survival and body condition of raccoons at the edge of the range. *Journal of Wildlife Management* 72 (2):389-395.
- Porter RH, Wyrick M, Pankey J (1978) Sibling recognition in spiny mice (*Acomys cahirinus*) *Behavioral Ecology and Sociobiology* 3 (1):61-68.
- Porter RH (1988) The ontogeny of sibling recognition in rodents: superfamily Muroidea. *Behavior Genetics* 18 (4):483-494.
- Poteaux C, Busquet N, Gouat P, Katona K, Baudoin C (2008) Socio-genetic structure of mound-building mice, *Mus spicilegus*, in autumn and early spring. *Biological Journal of the Linnean Society* 93 (4):689 - 699.
- Pucek Z, Jedrzejewski W, Jedrzejewska B, Pucek M (1993) Rodent population-dynamics in a primeval deciduous forest (Bialowieza National Park) in relation to weather, seed crop, and predation. *Acta Theriologica* 38 (2):199-232.
- Pulliam HR, Caraco T (1984) Living in groups: is there an optimal group size. In: Krebs JR, Davies NB (eds) *Behavioural Ecology: an Evolutionary Approach*, vol 2. Blackwell Scientific Publications, Oxford, pp 122-147
- Redman P, Selman C, Speakman JR (1999) Male short-tailed field voles (*Microtus agrestis*) build better insulated nests than females. *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology* 169 (8):581-587.
- Reichman OJ, Smith SC (1990) Burrows and burrowing behavior by mammals. In: Genoways HH (ed) *Current Mammalogy*. Plenum Press, New York, pp 197-244
- Reimer JD, Petras ML (1967) Breeding structure of the house mouse, *Mus musculus*, in a population cage. *Journal of Mammalogy* 48 (1):88-99.
- Roberts SC (1988) Social influences on vigilance in rabbits. *Animal Behaviour* 36:905-913.
- Rowe FP, Redfern R (1969) Aggressive behaviour in related and unrelated wild house mice (*Mus musculus* L.). *Annals of Applied Biology* 64 (3):425-431.

- Sachser N, Schwarz-Weig E, Keil A, Epplen JT (1999) Behavioural strategies, testis size, and reproductive success in two caviomorph rodents with different mating systems. *Behaviour* 136:1203-1217.
- Sage RD, Atchley WR, Capanna E (1993) House mice as models in systematic biology. *Systematic Biology* 42 (4):523-561.
- Saylor A, Salmon M (1969) Communal nursing in mice: influence of multiple mothers on the growth of the young. *Science* 164 (3885):1309-1310.
- Schug MD, Vessey SH, Underwood EM (1992) Paternal behavior in a natural population of white-footed mice (*Peromyscus leucopus*). *American Midland Naturalist* 127 (2):373-380.
- Serra J, Hurtado MJ, Le Négrate A, Féron C, Nowak R, Gouat P (2012) Behavioral differentiation during collective building in wild mice *Mus spicilegus*. *Behavioural Processes* 89 (3):292-298.
- Shapiro LE, Austin D, Ward SE, Dewsbury DA (1986) Familiarity and female mate choice in two species of voles (*Microtus ochrogaster* and *Microtus montanus*). *Animal Behaviour* 34:90-97.
- Sherman PW (1977) Nepotism and the evolution of alarm calls. *Science* 197 (4310):1246-1253.
- Simeonovska-Nikolova DM, Gerasimov S (2000) Seasonal changes of some population characteristics of *Mus spicilegus* Petenyi in North Bulgaria. *Acta Zoologica Bulgarica* 52:81-90.
- Simeonovska-Nikolova DM (2003) Social relationships and social structure of the mound-building mouse (*Mus spicilegus*) in intraspecific cage groups. *Acta Ethologica* 6:39-45.
- Simeonovska-Nikolova DM (2007) Spatial organization of the mound-building mouse *Mus spicilegus* in the region of northern Bulgaria. *Acta Zoologica Sinica* 53 (1):22-28.
- Sluyter F, van Oortmerssen GA, deRuiter AJH, Koolhaas JM (1996) Aggression in wild house mice: Current state of affairs. *Behavior Genetics* 26 (5):489-496.
- Sokolov VE, Kotenkova E, Lyalyukhina SI (1990) Biology of house and mound-building mice. Moskva: Nauka 207.
- Sokolov VE, Kotenkova EV, Michailenko AG (1998) *Mus spicilegus*. *Mammalian Species* 592:1-6.
- Springer SD, Gregory PA, Barrett GW (1981) Importance of social grouping on bioenergetics of the golden mouse, *Ochrotomys nuttalli*. *Journal of Mammalogy* 62 (3):628-630.
- StatSoft Inc. (2007) STATISTICA (data analysis software system). 8.0 edn. StatSoft Inc, Tulsa, Oklahoma.
- Stenseth NC, Lidicker WZ (1992) Animal dispersal: small mammals as models. Chapman & Hall, London
- Suchomelova E, Munclinger P, Frynta D (1998) New evidence of pseudosexual behaviour and female aggression in mice: neutral cage interactions in *Mus spicilegus* and *Mus spretus* (Rodentia : Muridae). *Folia Zoologica* 47 (4):241-247.

- Szenczi P, Bánszegi O, Dúcs A, Gedeon CI, Markó G, Németh I, Altbäcker V (2011) Morphology and function of communal mounds of overwintering mound-building mice (*Mus spicilegus*). *Journal of Mammalogy* 92 (4):852-860.
- Szenczi P, Kopcsó D, Bánszegi O, Altbäcker V (2012) The contribution of the vegetable material layer to the insulation capacities and water proofing of artificial *Mus spicilegus* mounds. *Mammalian Biology - Zeitschrift für Säugetierkunde* 77 (5):327-331.
- Szentirmai I, Komdeur J, Székely T (2005) What makes a nest-building male successful? Male behavior and female care in penduline tits. *Behavioral Ecology* 165 (6):994-1000.
- Therneau T (2011) coxme: mixed effects Cox models. 2.1-3 edn.
- Trainor BC, Marler CA (2001) Testosterone, paternal behavior, and aggression in the monogamous California mouse (*Peromyscus californicus*). *Hormones and Behavior* 40 (1):32-42.
- Trivers RL (1971) The evolution of reciprocal altruism. *The Quarterly Review of Biology* 46 (1):35-57.
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) *Sexual selection and the descent of man*. Aldine, Chicago, pp 136-176
- van Zegeren K (1980) Variation in aggressiveness and the regulation of numbers in House mouse populations. *Netherlands Journal of Zoology* 30 (4):635-770.
- Vander Wall SBV (1990) *Food hoarding in animals*. University of Chicago Press, Chicago
- Vleck D (1979) Energy cost of burrowing by the pocket gopher *Thomomys bottae*. *Physiological Zoology* 52 (2):122-136.
- Vom Saal FS, Franks P, Boechler M, Palanza P, Parmigiani S (1995) Nest defense and survival of offspring in highly aggressive wild Canadian female house mice. *Physiology & Behavior* 58 (4):669-678.
- Walters JR, Seyfarth RM (1986) Conflict and cooperation. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW (eds) *Primate societies*. University of Chicago Press, pp 306-317
- Wilkinson GS, Baker AEM (1988) Communal nesting among genetically similar house mice. *Ethology* 77:103-114.
- Wolff JO, Cicirello DM (1990) Mobility versus territoriality: alternative reproductive strategies in white-footed mice. *Animal Behaviour* 39:1222-1224.
- Wolff JO (1993) What is the role of adults in mammalian juvenile dispersal? *Oikos* 68 (1):173-176.
- Wolff JO (1994) More on juvenile dispersal in mammals. *Oikos* 71 (2):349-352.
- Wolff RJ (1985) Mating behaviour and female choice: their relation to social structure in wild caught House mice (*Mus musculus*) housed in a semi-natural environment. *Journal of Zoology* 207 (Sep):43-51.
- Wrangham RW (1981) Drinking competition in vervet monkeys. *Animal Behaviour* 29:904-910.

- Wrangham RW (1983) Social relationships in comparative perspective. In: Hinde R (ed) *Primate social relationships: an integrated approach*. Oxford: Blackwell, pp 325-334
- Zazula GD, Mathewes RW, Harestad AS (2006) Cache selection by arctic ground squirrels inhabiting Boreal-steppe meadows of southwest Yukon territory, Canada. *Arctic, Antarctic, and Alpine Research* 38 (4):631-638.
- Zhong W, Wang G, Zhoua Q, Wang G (2007) Communal food caches and social groups of Brandt's voles in the typical steppes of Inner Mongolia, China. *Journal of Arid Environments* 68 (3):398-407.

SUMMARY

This thesis deals with the role of the cooperatively built mounds of the mound-building mouse and with the social behaviour of that species using field studies and comparative laboratory experiments. I investigated the connection between the behavioural bases and ecological constraints of cooperative behaviour. I studied the morphology of the mounds, the diet of the mound-building mouse, and the effect of the mounds on the soil. In comparative tests with the house mouse I investigated the social and aggressive behaviour and the natal dispersal of the mound-building mouse.

I demonstrated:

- That the morphology of the mound do not but the location of the nest chamber depends on soil characteristics
- During their overwintering the mound-building mice do not consume the collected vegetable material
- The mound alters the temperature profile and water content of the soil beneath
- The collected plant material plays an important role in keeping the nest chamber dry
- During their overwintering the mortality rate of the mound-building mouse is low
- Both sexes of adult mound-building mouse shows elevated level of aggression toward strangers compared to the house mouse
- Both sexes of mound-building mouse remains tolerant toward their familiar siblings even after long separation
- The mound-building disperse at a later age than house mouse
- There was no sexual dimorphism in the natal dispersal of the mound-building mouse
- Weight at weaning do not affect the age of dispersal of the mound-building mouse

ÖSSZEFOGLALÓ

Disszertációm a güzü egerek által kooperatívan épített halom szerepével, és a güzü egér szociális viselkedésével foglalkozik terepi vizsgálatok és összehasonlító laboratóriumi kísérletek segítségével. Összefüggéseket kerestem a kooperációs viselkedési alapjai és azok ökológiai háttere között. Hogy megállapíthassuk az együttműködés eredményeként létrehozott halom szerepét, vizsgáltam a güzühalom morfológiai jellemzőit, a güzüegér táplálkozási szokásait, a güzühalom hatását a talaj hő és vízviszonyaira. Házi egérrel végzett összehasonlító kísérletekben tanulmányoztam a güzü egér szociális és agresszív viselkedését és fiatalkori diszperziójának mintázatát.

Ezek során kimutattam;

- Hogy a güzü-halmok külső morfológiája nem, de a fészekkamra elhelyezkedése függ a talaj tulajdonságaitól.
- A güzü-halom befolyásolja az alatta található talaj hőmérséklet és víz-viszonyait, védi a hőmérsékleti szélsőségektől és szárazon tartja a fészekkamrát.
- Az güzü egér áttelelése közben nem, vagy alig fogyasztja a halomban felhalmozott növényi anyagokat.
- A güzü-halomban felhalmozott növényi részek jelentősen hozzájárulnak a halom szigetelő tulajdonságához, jelentős szerepük a fészekkamra szárazon tartásában.
- A güzü egerek áttelelése során alacsony a mortalitás
- A felnőtt güzü egerek mindkét neme a házi egerekkel összehasonlítva fokozottan agresszív ismeretlen fajtársaival szemben.
- A güzü egerek mindkét neme ismert alomtársaival még hosszú szeparáció után is toleráns.
- A güzü egér a házi egérrel összehasonlítva csökkent fiatalkori elvándorlást mutat
- A güzü egerek esetében nem figyelhető meg ivari dimorfizmus a fiatalkori elvándorlásban
- A güzü egér esetében a fiatalkori elvándorlást nem befolyásolja az elválasztáskori súly.

LIST OF RELATED PUBLICATIONS

- Szenczi, P., Bánszegi, O., Dúcs, A., Gedeon, C. I., Markó, G., Németh, I., Altbäcker, V. (2011). Morphology and function of communal mounds of overwintering mound-building mice (*Mus spicilegus*). *Journal of Mammalogy*, 92 (4), 852-860. doi: 10.1644/10-mamm-a-258.1
- Szenczi, P., Kopcsó, D., Bánszegi, O., Altbäcker, V. (2012). The contribution of the vegetable material layer to the insulation capacities and water proofing of artificial *Mus spicilegus* mounds. *Mammalian Biology - Zeitschrift für Säugetierkunde* 77 (5), 327–331. doi: 10.1016/j.mambio.2012.03.003
- Szenczi, P., Bánszegi, O., Groó, Z., Altbäcker, V. (2012). Development of the social behavior of two mice species with contrasting social systems. *Aggressive Behavior*, 38 (4), 288-297. doi: 10.1002/ab.21431

ACKNOWLEDGEMENTS

Firstly, I wish to express my gratitude to Vilmos Altbäcker for starting the mound-building mouse project and for all the valuable things he taught me during the years. His enthusiasm toward nature, his unique knowledge and experiences which he was always willing to share greatly influenced my scientific and personal views (f16 at 1/250 for greater dof).

I would also like to thank everyone with whom I worked together on any ground-squirrel, rabbit or mouse project, especially Anita Dúcs, Csongor Gedeon, Zita Groó, Dániel Kopcsó, István Németh, Gábor Markó. Spending time with them was not only edifying but also very entertaining.

I thank all the colleagues at the Ethology Department especially András Kosztolányi for his advices concerning the statistical methods and for Károly Csizmadia for his help in the work with the animals.

Not forgetting my friends, Patricia Dévényi and Márta Antal for spending many precious hours with correcting the English of my manuscripts. Their contribution was invaluable.

I am particularly grateful for the life-long support of my family, for believing me.

For Oxána

For everything